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(54) Title: HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE

(57) Abstract: The invention provides improved agents and methods for treatment of diseases associated with amyloid deposits of Ab in the brain of a patient. Preferred agents include antibodies, e.g., humanized antibodies.

HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE**Related Applications**

This application claims the benefit of prior-filed provisional patent
5 application U.S. Serial No. 60/474,654 (filed May 30, 2003) entitled "Humanized Antibodies That Recognize Beta Amyloid Peptide". The entire content of the above-referenced application is incorporated herein by reference.

Background of the Invention

10 Alzheimer's disease (AD) is a progressive disease resulting in senile dementia. *See generally* Selkoe, *TINS* 16:403 (1993); Hardy *et al.*, WO 92/13069; Selkoe, *J. Neuropathol. Exp. Neurol.* 53:438 (1994); Duff *et al.*, *Nature* 373:476 (1995); Games *et al.*, *Nature* 373:523 (1995). Broadly speaking, the disease falls into two categories: late onset, which occurs in old age (65 + years) and early onset, which
15 develops well before the senile period, *i.e.*, between 35 and 60 years. In both types of disease, the pathology is the same but the abnormalities tend to be more severe and widespread in cases beginning at an earlier age. The disease is characterized by at least two types of lesions in the brain, neurofibrillary tangles and senile plaques.
Neurofibrillary tangles are intracellular deposits of microtubule associated tau protein
20 consisting of two filaments twisted about each other in pairs. Senile plaques (*i.e.*, amyloid plaques) are areas of disorganized neuropil up to 150 μm across with extracellular amyloid deposits at the center which are visible by microscopic analysis of sections of brain tissue. The accumulation of amyloid plaques within the brain is also associated with Down's syndrome and other cognitive disorders.

25 The principal constituent of the plaques is a peptide termed A β or β -amyloid peptide. A β peptide is a 4-kDa internal fragment of 39-43 amino acids of a larger transmembrane glycoprotein named protein termed amyloid precursor protein (APP). As a result of proteolytic processing of APP by different secretase enzymes, A β is primarily found in both a short form, 40 amino acids in length, and a long form,
30 ranging from 42-43 amino acids in length. Part of the hydrophobic transmembrane domain of APP is found at the carboxy end of A β , and may account for the ability of A β to aggregate into plaques, particularly in the case of the long form. Accumulation of amyloid plaques in the brain eventually leads to neuronal cell death. The physical

symptoms associated with this type of neural deterioration characterize Alzheimer's disease.

Several mutations within the APP protein have been correlated with the presence of Alzheimer's disease. See, e.g., Goate *et al.*, *Nature* 349:704 (1991) (5) (valine⁷¹⁷ to isoleucine); Chartier Harlan *et al.* *Nature* 353:844 (1991) (valine⁷¹⁷ to glycine); Murrell *et al.*, *Science* 254:97 (1991) (valine⁷¹⁷ to phenylalanine); Mullan *et al.*, *Nature Genet.* 1:345 (1992) (a double mutation changing lysine⁵⁹⁵-methionine⁵⁹⁶ to asparagine⁵⁹⁵-leucine⁵⁹⁶). Such mutations are thought to cause Alzheimer's disease by increased or altered processing of APP to A β , particularly processing of APP to 10 increased amounts of the long form of A β (*i.e.*, A β 1-42 and A β 1-43). Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of long form A β (see Hardy, *TINS* 20: 154 (1997)).

Mouse models have been used successfully to determine the significance 15 of amyloid plaques in Alzheimer's (Games *et al.*, *supra*, Johnson-Wood *et al.*, *Proc. Natl. Acad. Sci. USA* 94:1550 (1997)). In particular, when PDAPP transgenic mice, (which express a mutant form of human APP and develop Alzheimer's disease at a young age), are injected with the long form of A β , they display both a decrease in the progression of Alzheimer's and an increase in antibody titers to the A β peptide (Schenk 20 *et al.*, *Nature* 400, 173 (1999)). The observations discussed above indicate that A β , particularly in its long form, is a causative element in Alzheimer's disease.

Accordingly, there exists the need for new therapies and reagents for the treatment of Alzheimer's disease, in particular, therapies and reagents capable of effecting a therapeutic benefit at physiologic (*e.g.*, non-toxic) doses.

25

Summary of the Invention

The present invention features new immunological reagents, in particular, therapeutic antibody reagents for the prevention and treatment of amyloidogenic disease (e.g., Alzheimer's disease). The invention is based, at least in part, on the identification 30 and characterization of a monoclonal antibody, 12A11, that specifically binds to A β peptide and is effective at reducing plaque burden associated with amyloidogenic disorders. Structural and functional analysis of this antibody leads to the design of various humanized antibodies for prophylactic and/or therapeutic use. In particular, the

invention features humanization of the variable regions of this antibody and, accordingly, provides for humanized immunoglobulin or antibody chains, intact humanized immunoglobulins or antibodies, and functional immunoglobulin or antibody fragments, in particular, antigen binding fragments, of the featured antibody.

5 Polypeptides comprising the complementarity determining regions (CDRs) of the featured monoclonal antibody are also disclosed, as are polynucleotide reagents, vectors and host cells suitable for encoding said polypeptides.

Methods for treating amyloidogenic diseases or disorders (e.g., Alzheimer's disease) are disclosed, as are pharmaceutical compositions and kits for use 10 in such applications.

Also featured are methods of identifying residues within the featured monoclonal antibodies which are important for proper immunologic function and for identifying residues which are amenable to substitution in the design of humanized antibodies having improved binding affinities and/or reduced immunogenicity, when 15 used as therapeutic reagents.

Also featured are antibodies (e.g., humanized antibodies) having altered effector functions, and therapeutic uses thereof.

Brief Description of the Drawings

20 *Figure 1* graphically depicts results from an experiment which examines the effectiveness of various antibodies, including 12A11, at clearing A β plaques in an *ex vivo* phagocytosis assay.

Figure 2A graphically depicts results from an experiment which examines the effectiveness of various antibodies, including 12A11, at reducing total A β levels.

25 The bars represent median values, and the dashed horizontal line indicates the control level. *Figure 2B* graphically depicts results from an experiment which analyzes the effectiveness of various antibodies, including 12A11, at reducing neuritic dystrophy. The bars represent median values, and the dashed horizontal line indicates the control level. Data are shown for individual animals and expressed as the percentage of neuritic dystrophy relative to the mean of the control (set at 100%).

30 *Figure 3A* depicts a DNA sequence including the murine 12A11 VL chain sequence and the deduced amino acid sequence for the VL chain (SEQ ID NOS: 5 and 2, respectively). Mature VL chain is indicated by a solid black bar. CDRs are

indicated by open bars. *Figure 3B* depicts a DNA sequence including the murine 12A11 VH chain sequence and the deduced amino acid sequence for the VH chain (SEQ ID NOs: 6 and 3, respectively). Mature VH chain is indicated by a solid black bar. CDRs are indicated by open bars. DNA sequences include cloning sites and Kozak sequences upstream of coding sequences) and splice and cloning sequences (downstream).

5 *Figure 4* graphically depicts the ELISA results from an experiment measuring the binding of chimeric 12A11, chimeric and humanized 3D6, and chimeric and humanized 12B4 to A β 1-42.

10 *Figure 5A* depicts an alignment of the amino acid sequences of the light chain of murine (or chimeric) 12A11 (SEQ ID NO:2), humanized 12A11 (mature peptide, SEQ ID NO:7), GenBank BAC01733 (SEQ ID NO: 8) and germline A19 (X63397, SEQ ID NO: 9) antibodies. CDR regions are boxed. Packing residues are underlined. Numbered from the first methionine, not Kabat numbering. *Figure 5B* depicts an alignment of the amino acid sequences of the heavy chain of murine (or 15 chimeric) 12A11 (SEQ ID NO:4), humanized 12aA11 (version 1) (mature peptide, SEQ ID NO:10), GenBank AAA69734 (SEQ ID NO:11), and germline GenBank 567123 antibodies (SEQ ID NO:12). Packing residues are underlined, canonical residues are in solid fill and vernier residues are in dotted fill. Numbered from the first methionine, not Kabat numbering.

20 *Figure 6A-B* depicts an alignment of the amino acid sequences of the heavy chains of humanized 12A11 v1 (SEQ ID NO:10), v2 (SEQ ID NO:13), v2.1 (SEQ ID NO:14), v3 (SEQ ID NO:15), v4.1 (SEQ ID NO:16), v4.2 (SEQ ID NO:17), v4.3 (SEQ ID NO:18), v4.4 (SEQ ID NO:19), v5.1 (SEQ ID NO:20), v5.2 (SEQ ID NO:21), v5.3 (SEQ ID NO:22), v5.4 (SEQ ID NO:23), v5.5 (SEQ ID NO:24), v5.6 (SEQ ID NO:25), v6.1 (SEQ ID NO:26), v6.2 (SEQ ID NO:27), v6.3 (SEQ ID NO:28), v6.4 (SEQ ID NO:29), v7 (SEQ ID NO:30) and v8 (SEQ ID NO:31). *Figure 6C* sets forth the backmutations made in humanized 12A11 v1 to v8.

25 *Figure 7* depicts the results from an aggregated A β (1-42) ELISA comparing chimeric 12A11, humanized 12A11 v1, humanized 12A11 v2, humanized 12A11 v2.1, and humanized 12A11 v3.

30 *Figure 8* depicts the results of a competitive A β 1-42 ELISA binding assay comparing murine 12A11, chimeric 12A11 and h12A11 v1.

Detailed Description of the Invention

The present invention features new immunological reagents and methods for preventing or treating Alzheimer's disease or other amyloidogenic diseases. The invention is based, at least in part, on the characterization of a monoclonal immunoglobulin, 12A11, effective at binding beta amyloid protein (A β) (e.g., binding soluble and/or aggregated A β), mediating phagocytosis (e.g., of aggregated A β), reducing plaque burden and/or reducing neuritic dystrophy (e.g., in a patient). The invention is further based on the determination and structural characterization of the primary and secondary structure of the variable light and heavy chains of the 12A11 immunoglobulin and the identification of residues important for activity and immunogenicity.

Immunoglobulins are featured which include a variable light and/or variable heavy chain of the 12A11 monoclonal immunoglobulin described herein. Preferred immunoglobulins, e.g., therapeutic immunoglobulins, are featured which include a humanized variable light and/or humanized variable heavy chain. Preferred variable light and/or variable heavy chains include a complementarity determining region (CDR) from the 12A11 immunoglobulin (e.g., donor immunoglobulin) and variable framework regions from or substantially from a human acceptor immunoglobulin. The phrase "substantially from a human acceptor immunoglobulin" means that the majority or key framework residues are from the human acceptor sequence, allowing however, for substitution of residues at certain positions with residues selected to improve activity of the humanized immunoglobulin (e.g., alter activity such that it more closely mimics the activity of the donor immunoglobulin) or selected to decrease the immunogenicity of the humanized immunoglobulin.

In one embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 12A11 variable region complementarity determining regions (CDRs) (i.e., includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:2 or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a variable framework region from a human acceptor immunoglobulin light or heavy chain sequence, optionally having at least one residue of the framework residue backmutated to a corresponding murine residue, wherein said backmutation does not substantially affect the ability of the chain to direct A β binding.

In one embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 12A11 variable region complementarity determining regions (CDRs) (*i.e.*, includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:2 or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence, optionally having at least one residue of the framework residue backmutated to a corresponding murine residue, wherein said backmutation does not substantially affect the ability of the chain to direct A β binding.

10 In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 12A11 variable region complementarity determining regions (CDRs) (*e.g.*, includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:2 and/or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ 15 ID NO:4), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence, optionally having at least one framework residue substituted with the corresponding amino acid residue from the mouse 12A11 light or heavy chain variable region sequence, where the framework residue is selected from the group consisting of (a) a residue that non-covalently binds antigen directly; (b) a residue adjacent to a CDR; (c) a CDR-interacting residue (*e.g.*, identified by modeling the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); and (d) a residue participating in the VL-VH interface.

20 In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 12A11 variable region CDRs and 25 variable framework regions from a human acceptor immunoglobulin light or heavy chain sequence, optionally having at least one framework residue substituted with the corresponding amino acid residue from the mouse 12A11 light or heavy chain variable region sequence, where the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the variable region, for example a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR 30

residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, or a glycosylation site residue on the surface of the structural model.

In another embodiment, the invention features, in addition to the substitutions described above, a substitution of at least one rare human framework residue. For example, a rare residue can be substituted with an amino acid residue which is common for human variable chain sequences at that position. Alternatively, a rare residue can be substituted with a corresponding amino acid residue from a homologous germline variable chain sequence.

In another embodiment, the invention features a humanized immunoglobulin that includes a light chain and a heavy chain, as described above, or an antigen-binding fragment of said immunoglobulin. In an exemplary embodiment, the humanized immunoglobulin binds (e.g., specifically binds) to beta amyloid peptide (A β) with a binding affinity of at least 10^7 M $^{-1}$, 10^8 M $^{-1}$, or 10^9 M $^{-1}$. In another embodiment, the immunoglobulin or antigen binding fragment includes a heavy chain having isotype $\gamma 1$. In another embodiment, the immunoglobulin or antigen binding fragment binds (e.g., specifically binds) to either or both soluble beta amyloid peptide (A β) and aggregated A β . In another embodiment, the immunoglobulin or antigen binding fragment captures soluble A β (e.g., soluble A $\beta 1-42$). In another embodiment, the immunoglobulin or antigen binding fragment mediates phagocytosis (e.g., induces phagocytosis) of beta amyloid peptide (A β). In yet another embodiment, the immunoglobulin or antigen binding fragment crosses the blood-brain barrier in a subject. In yet another embodiment, the immunoglobulin or antigen binding fragment reduces either or both beta amyloid peptide (A β) burden and neuritic dystrophy in a subject.

In another embodiment, the invention features chimeric immunoglobulins that include 12A11 variable regions (e.g., the variable region sequences set forth as SEQ ID NO:2 or SEQ ID NO:4). In yet another embodiment, the immunoglobulin, or antigen-binding fragment thereof, further includes constant regions from IgG1.

The immunoglobulins described herein are particularly suited for use in therapeutic methods aimed at preventing or treating amyloidogenic diseases. In one embodiment, the invention features a method of preventing or treating an amyloidogenic disease (e.g., Alzheimer's disease) that involves administering to the patient an effective dosage of a humanized immunoglobulin as described herein. In another embodiment, the invention features pharmaceutical compositions that include a humanized

immunoglobulin as described herein and a pharmaceutical carrier. Also featured are isolated nucleic acid molecules, vectors and host cells for producing the immunoglobulins or immunoglobulin fragments or chains described herein, as well as methods for producing said immunoglobulins, immunoglobulin fragments or

5 immunoglobulin chains

The present invention further features a method for identifying 12A11 residues amenable to substitution when producing a humanized 12A11 immunoglobulin, respectively. For example, a method for identifying variable framework region residues amenable to substitution involves modeling the three-dimensional structure of a 12A11
10 variable region on a solved homologous immunoglobulin structure and analyzing said model for residues capable of affecting 12A11 immunoglobulin variable region conformation or function, such that residues amenable to substitution are identified. The invention further features use of the variable region sequence set forth as SEQ ID NO:2 or SEQ ID NO:4, or any portion thereof, in producing a three-dimensional image of a
15 12A11 immunoglobulin, 12A11 immunoglobulin chain, or domain thereof.

The present invention further features immunoglobulins having altered effector function, such as the ability to bind effector molecules, for example, complement or a receptor on an effector cell. In particular, the immunoglobulin of the invention has an altered constant region, *e.g.*, Fc region, wherein at least one amino acid residue in the Fc region has been replaced with a different residue or side chain. In one embodiment, the modified immunoglobulin is of the IgG class, comprises at least one amino acid residue replacement in the Fc region such that the immunoglobulin has an altered effector function, *e.g.*, as compared with an unmodified immunoglobulin. In particular embodiments, the immunoglobulin of the invention has an altered effector
20 function such that it is less immunogenic (*e.g.*, does not provoke undesired effector cell activity, lysis, or complement binding), has improved amyloid clearance properties, and/or has a desirable half-life.
25

Prior to describing the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms to be used hereinafter.

30 The term "immunoglobulin" or "antibody" (used interchangeably herein) refers to a protein having a basic four-polypeptide chain structure consisting of two heavy and two light chains, said chains being stabilized, for example, by interchain disulfide bonds, which has the ability to specifically bind antigen. The term "single-

chain immunoglobulin" or "single-chain antibody" (used interchangeably herein) refers to a protein having a two-polypeptide chain structure consisting of a heavy and a light chain, said chains being stabilized, for example, by interchain peptide linkers, which has the ability to specifically bind antigen. The term "domain" refers to a globular region of

5 a heavy or light chain polypeptide comprising peptide loops (e.g., comprising 3 to 4 peptide loops) stabilized, for example, by β -pleated sheet and/or intrachain disulfide bond. Domains are further referred to herein as "constant" or "variable", based on the relative lack of sequence variation within the domains of various class members in the case of a "constant" domain, or the significant variation within the domains of various

10 class members in the case of a "variable" domain. Antibody or polypeptide "domains" are often referred to interchangeably in the art as antibody or polypeptide "regions". The "constant" domains of an antibody light chain are referred to interchangeably as "light chain constant regions", "light chain constant domains", "CL" regions or "CL" domains. The "constant" domains of an antibody heavy chain are referred to

15 interchangeably as "heavy chain constant regions", "heavy chain constant domains", "CH" regions or "CH" domains). The "variable" domains of an antibody light chain are referred to interchangeably as "light chain variable regions", "light chain variable domains", "VL" regions or "VL" domains). The "variable" domains of an antibody heavy chain are referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "VH" regions or "VH" domains).

20

The term "region" can also refer to a part or portion of an antibody chain or antibody chain domain (e.g., a part or portion of a heavy or light chain or a part or portion of a constant or variable domain, as defined herein), as well as more discrete parts or portions of said chains or domains. For example, light and heavy chains or light

25 and heavy chain variable domains include "complementarity determining regions" or "CDRs" interspersed among "framework regions" or "FRs", as defined herein.

Immunoglobulins or antibodies can exist in monomeric or polymeric form, for example, IgM antibodies which exist in pentameric form and/or IgA antibodies which exist in monomeric, dimeric or multimeric form. The term "fragment" refers to a

30 part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. Fragments can be obtained via chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant means. Exemplary fragments include

Fab, Fab', F(ab')₂, Fabc and/or Fv fragments. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (*i.e.*, with the intact antibody from which they were derived) for antigen binding (*i.e.*, specific binding).

5 The term "conformation" refers to the tertiary structure of a protein or polypeptide (*e.g.*, an antibody, antibody chain, domain or region thereof). For example, the phrase "light (or heavy) chain conformation" refers to the tertiary structure of a light (or heavy) chain variable region, and the phrase "antibody conformation" or "antibody fragment conformation" refers to the tertiary structure of an antibody or fragment
10 thereof.

 "Specific binding" of an antibody means that the antibody exhibits appreciable affinity for a particular antigen or epitope and, generally, does not exhibit significant crossreactivity. In exemplary embodiments, the antibody exhibits no crossreactivity (*e.g.*, does not crossreact with non-A β peptides or with remote epitopes 15 on A β). "Appreciable" or preferred binding includes binding with an affinity of at least 10^6 , 10^7 , 10^8 , 10^9 M $^{-1}$, or 10^{10} M $^{-1}$. Affinities greater than 10^7 M $^{-1}$, preferably greater than 10^8 M $^{-1}$ are more preferred. Values intermediate of those set forth herein are also intended to be within the scope of the present invention and a preferred binding affinity can be indicated as a range of affinities, for example, 10^6 to 10^{10} M $^{-1}$, preferably 10^7 to 20 10^{10} M $^{-1}$, more preferably 10^8 to 10^{10} M $^{-1}$. An antibody that "does not exhibit significant crossreactivity" is one that will not appreciably bind to an undesirable entity (*e.g.*, an undesirable proteinaceous entity). For example, an antibody that specifically binds to A β will appreciably bind A β but will not significantly react with non-A β proteins or peptides (*e.g.*, non-A β proteins or peptides included in plaques). An antibody specific 25 for a particular epitope will, for example, not significantly crossreact with remote epitopes on the same protein or peptide. Specific binding can be determined according to any art-recognized means for determining such binding. Preferably, specific binding is determined according to Scatchard analysis and/or competitive binding assays.

 Binding fragments are produced by recombinant DNA techniques, or by 30 enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', F(ab')₂, Fabc, Fv, single chains, and single-chain antibodies. Other than "bispecific" or "bifunctional" immunoglobulins or antibodies, an immunoglobulin or antibody is understood to have each of its binding sites identical. A "bispecific" or

“bifunctional antibody” is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, 5 *J. Immunol.* 148, 1547-1553 (1992).

The term “humanized immunoglobulin” or “humanized antibody” refers to an immunoglobulin or antibody that includes at least one humanized immunoglobulin or antibody chain (*i.e.*, at least one humanized light or heavy chain). The term “humanized immunoglobulin chain” or “humanized antibody chain” (*i.e.*, a “humanized 10 immunoglobulin light chain” or “humanized immunoglobulin heavy chain”) refers to an immunoglobulin or antibody chain (*i.e.*, a light or heavy chain, respectively) having a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) (*e.g.*, at least one CDR, preferably two CDRs, more preferably three CDRs) substantially from a 15 non-human immunoglobulin or antibody, and further includes constant regions (*e.g.*, at least one constant region or portion thereof, in the case of a light chain, and preferably three constant regions in the case of a heavy chain). The term “humanized variable region” (*e.g.*, “humanized light chain variable region” or “humanized heavy chain variable region”) refers to a variable region that includes a variable framework region 20 substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) substantially from a non-human immunoglobulin or antibody.

The phrase “substantially from a human immunoglobulin or antibody” or “substantially human” means that, when aligned to a human immunoglobulin or 25 antibody amino sequence for comparison purposes, the region shares at least 80-90%, 90-95%, or 95-99% identity (*i.e.*, local sequence identity) with the human framework or constant region sequence, allowing, for example, for conservative substitutions, consensus sequence substitutions, germline substitutions, backmutations, and the like. The introduction of conservative substitutions, consensus sequence substitutions, 30 germline substitutions, backmutations, and the like, is often referred to as “optimization” of a humanized antibody or chain. The phrase “substantially from a non-human immunoglobulin or antibody” or “substantially non-human” means having an immunoglobulin or antibody sequence at least 80-95%, preferably at least 90-95%, more

preferably, 96%, 97%, 98%, or 99% identical to that of a non-human organism, e.g., a non-human mammal.

Accordingly, all regions or residues of a humanized immunoglobulin or antibody, or of a humanized immunoglobulin or antibody chain, except possibly the 5 CDRs, are substantially identical to the corresponding regions or residues of one or more native human immunoglobulin sequences. The term "corresponding region" or "corresponding residue" refers to a region or residue on a second amino acid or nucleotide sequence which occupies the same (*i.e.*, equivalent) position as a region or residue on a first amino acid or nucleotide sequence, when the first and second 10 sequences are optimally aligned for comparison purposes.

The term "significant identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 50-60% sequence identity, preferably at least 60-70% sequence identity, more preferably at least 70-80% sequence identity, more preferably at least 80-15 90% identity, even more preferably at least 90-95% identity, and even more preferably at least 95% sequence identity or more (e.g., 99% sequence identity or more). The term "substantial identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80-90% sequence identity, preferably at least 90-95% sequence identity, and more 20 preferably at least 95% sequence identity or more (e.g., 99% sequence identity or more). For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence 25 comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), 30 by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package,

Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally* Ausubel *et al.*, Current Protocols in Molecular Biology). One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (publicly accessible through the National Institutes of Health NCBI internet server). Typically, default program parameters can be used to perform the sequence comparison, although customized parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

Preferably, residue positions which are not identical differ by conservative amino acid substitutions. For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows:

15 Group I (hydrophobic sidechains): leu, met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

Preferably, humanized immunoglobulins or antibodies bind antigen with an affinity that is within a factor of three, four, or five of that of the corresponding non-humanized antibody. For example, if the nonhumanized antibody has a binding affinity of 10^9 M^{-1} , humanized antibodies will have a binding affinity of at least $3 \times 10^9 \text{ M}^{-1}$, $4 \times 10^9 \text{ M}^{-1}$ or $5 \times 10^9 \text{ M}^{-1}$. When describing the binding properties of an immunoglobulin or antibody chain, the chain can be described based on its ability to "direct antigen (*e.g.*, A β) binding". A chain is said to "direct antigen binding" when it confers upon an intact immunoglobulin or antibody (or antigen binding fragment thereof) a specific binding property or binding affinity. A mutation (*e.g.*, a backmutation) is said to substantially affect the ability of a heavy or light chain to direct antigen binding if it affects (*e.g.*, decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by at least an order of magnitude

compared to that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation. A mutation “does not substantially affect (e.g., decrease) the ability of a chain to direct antigen binding” if it affects (e.g., decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by only a factor of two, three, or four of that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation.

The term “chimeric immunoglobulin” or antibody refers to an immunoglobulin or antibody whose variable regions derive from a first species and whose constant regions derive from a second species. Chimeric immunoglobulins or antibodies can be constructed, for example by genetic engineering, from immunoglobulin gene segments belonging to different species. The terms “humanized immunoglobulin” or “humanized antibody” are not intended to encompass chimeric immunoglobulins or antibodies, as defined *infra*. Although humanized immunoglobulins or antibodies are chimeric in their construction (*i.e.*, comprise regions from more than one species of protein), they include additional features (*i.e.*, variable regions comprising donor CDR residues and acceptor framework residues) not found in chimeric immunoglobulins or antibodies, as defined herein.

An “antigen” is an entity (e.g., a proteinaceous entity or peptide) to which an antibody specifically binds.

The term “epitope” or “antigenic determinant” refers to a site on an antigen to which an immunoglobulin or antibody (or antigen binding fragment thereof) specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66, G. E. Morris, Ed. (1996).

Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, *i.e.*, a competitive binding assay. Competitive binding is determined in an assay in which the immunoglobulin under test inhibits specific binding of a reference antibody to a common antigen, such as A β . Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see Stahli *et al.*, *Methods in Enzymology* 9:242 (1983)); solid phase direct biotin-avidin EIA (see Kirkland *et al.*, *J. Immunol.* 137:3614 (1986)); solid phase direct labeled assay, solid phase direct labeled sandwich assay (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1988)); solid phase direct label RIA using I-125 label (see Morel *et al.*, *Mol. Immunol.* 25(1):7 (1988)); solid phase direct biotin-avidin EIA (Cheung *et al.*, *Virology* 176:546 (1990)); and direct labeled RIA. (Moldenhauer *et al.*, *Scand. J. Immunol.* 32:77 (1990)).

Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70% 70-75% or more.

An epitope is also recognized by immunologic cells, for example, B cells and/or T cells. Cellular recognition of an epitope can be determined by *in vitro* assays that measure antigen-dependent proliferation, as determined by 3 H-thymidine incorporation, by cytokine secretion, by antibody secretion, or by antigen-dependent killing (cytotoxic T lymphocyte assay).

Exemplary epitopes or antigenic determinants can be found within the human amyloid precursor protein (APP), but are preferably found within the A β peptide of APP. Multiple isoforms of APP exist, for example APP⁶⁹⁵ APP⁷⁵¹ and APP⁷⁷⁰. Amino acids within APP are assigned numbers according to the sequence of the APP⁷⁷⁰ isoform (see *e.g.*, GenBank Accession No. P05067). A β (also referred to herein as beta amyloid peptide and A-beta) peptide is an approximately 4-kDa internal fragment of 39-43 amino acids of APP (A β 39, A β 40, A β 41, A β 42 and A β 43). A β 40, for example,

consists of residues 672-711 of APP and A β 42 consists of residues 673-713 of APP. As a result of proteolytic processing of APP by different secretase enzymes *iv vivo* or *in situ*, A β is found in both a “short form”, 40 amino acids in length, and a “long form”, ranging from 42-43 amino acids in length. Preferred epitopes or antigenic determinants, 5 as described herein, are located within the N-terminus of the A β peptide and include residues within amino acids 1-10 of A β , preferably from residues 1-3, 1-4, 1-5, 1-6, 1-7 or 3-7 of A β 42. Additional referred epitopes or antigenic determinants include residues 2-4, 5, 6, 7 or 8 of A β , residues 3-5, 6, 7, 8 or 9 of A β , or residues 4-7, 8, 9 or 10 of A β 42. When an antibody is said to bind to an epitope within specified residues, such as 10 A β 3-7, what is meant is that the antibody specifically binds to a polypeptide containing the specified residues (*i.e.*, A β 3-7 in this example). Such an antibody does not necessarily contact every residue within A β 3-7. Nor does every single amino acid substitution or deletion within A β 3-7 necessarily significantly affect binding affinity.

The term “amyloidogenic disease” includes any disease associated with 15 (or caused by) the formation or deposition of insoluble amyloid fibrils. Exemplary amyloidogenic diseases include, but are not limited to systemic amyloidosis, Alzheimer’s disease, mature onset diabetes, Parkinson’s disease, Huntington’s disease, fronto-temporal dementia, and the prion-related transmissible spongiform encephalopathies (kuru and Creutzfeldt-Jacob disease in humans and scrapie and BSE in 20 sheep and cattle, respectively). Different amyloidogenic diseases are defined or characterized by the nature of the polypeptide component of the fibrils deposited. For example, in subjects or patients having Alzheimer’s disease, β -amyloid protein (*e.g.*, wild-type, variant, or truncated β -amyloid protein) is the characterizing polypeptide component of the amyloid deposit. Accordingly, Alzheimer’s disease is an example of a 25 “disease characterized by deposits of A β ” or a “disease associated with deposits of A β ”, *e.g.*, in the brain of a subject or patient. The terms “ β -amyloid protein”, “ β -amyloid peptide”, “ β -amyloid”, “A β ” and “A β peptide” are used interchangeably herein.

An “immunogenic agent” or “immunogen” is capable of inducing an immunological response against itself on administration to a mammal, optionally in 30 conjunction with an adjuvant.

The term “treatment” as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a

therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

5 The term “effective dose” or “effective dosage” is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term “therapeutically effective dose” is defined as an amount sufficient to cure or at least partially arrest the disease and its complications in a patient already suffering from the disease. Amounts effective for this use will depend upon the severity of the infection
10 and the general state of the patient’s own immune system.

The term “patient” includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

“Soluble” or “dissociated” A β refers to non-aggregating or disaggregated A β polypeptide, including monomeric soluble as well as oligomeric soluble A β
15 polypeptide (e.g., soluble A β dimers, trimers, and the like). “Insoluble” A β refers to aggregating A β polypeptide, for example, A β held together by noncovalent bonds. A β (e.g., A β 42) is believed to aggregate, at least in part, due to the presence of hydrophobic residues at the C-terminus of the peptide (part of the transmembrane domain of APP).
Soluble A β can be found *in vivo* in biological fluids such as cerebrospinal fluid and/or
20 serum. Alternatively, soluble A β can be prepared by dissolving lyophilized peptide in neat DMSO with sonication. The resulting solution is centrifuged (e.g., at 14,000x g,
4°C, 10 minutes) to remove any insoluble particulates.

The term “effector function” refers to an activity that resides in the Fc region of an antibody (e.g., an IgG antibody) and includes, for example, the ability of the
25 antibody to bind effector molecules such as complement and/or Fc receptors, which can control several immune functions of the antibody such as effector cell activity, lysis, complement-mediated activity, antibody clearance, and antibody half-life.

The term “effector molecule” refers to a molecule that is capable of binding to the Fc region of an antibody (e.g., an IgG antibody) including, but not limited
30 to, a complement protein or a Fc receptor.

The term "effector cell" refers to a cell capable of binding to the Fc portion of an antibody (*e.g.*, an IgG antibody) typically *via* an Fc receptor expressed on the surface of the effector cell including, but not limited to, lymphocytes, *e.g.*, antigen presenting cells and T cells.

- 5 The term "Fc region" refers to a C-terminal region of an IgG antibody, in particular, the C-terminal region of the heavy chain(s) of said IgG antibody. Although the boundaries of the Fc region of an IgG heavy chain can vary slightly, a Fc region is typically defined as spanning from about amino acid residue Cys226 to the carboxyl-terminus of an IgG heavy chain(s).
- 10 The term "Kabat numbering" unless otherwise stated, is defined as the numbering of the residues in, *e.g.*, an IgG heavy chain antibody using the EU index as in Kabat *et al.* (*Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)), expressly incorporated herein by reference.
- 15 The term "Fc receptor" or "FcR" refers to a receptor that binds to the Fc region of an antibody. Typical Fc receptors which bind to an Fc region of an antibody (*e.g.*, an IgG antibody) include, but are not limited to, receptors of the Fc γ RI, Fc γ RII, and Fc γ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc receptors are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995).

I. Immunological and Therapeutic Reagents

- 25 Immunological and therapeutic reagents of the invention comprise or consist of immunogens or antibodies, or functional or antigen binding fragments thereof, as defined herein. The basic antibody structural unit is known to comprise a tetramer of subunits. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or 30 more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda and are about 230 residues in length. Heavy chains are classified as gamma (γ), mu (μ), alpha (α), delta (δ), or epsilon (ϵ), are about 450-600 residues in length, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Both heavy and light chains are folded into domains. The term "domain" refers to a globular region of a protein, for example, an immunoglobulin or antibody. Immunoglobulin or antibody domains include, for example, three or four peptide loops stabilized by β -pleated sheet and an interchain disulfide bond. Intact light chains have, for example, two domains (V_L and C_L) and intact heavy chains have, for example, four or five domains (V_H , C_H1 , C_H2 , and C_H3).

Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. (See generally, *Fundamental Immunology* (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989), Ch. 7, incorporated by reference in its entirety for all purposes).

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. Naturally-occurring chains or recombinantly produced chains can be expressed with a leader sequence which is removed during cellular processing to produce a mature chain. Mature chains can also be recombinantly produced having a non-naturally occurring leader sequence, for example, to enhance secretion or alter the processing of a particular chain of interest.

The CDRs of the two mature chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. "FR4" also is referred to in the art as the D/J region of the variable heavy chain and the J region of the variable light chain. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991). An alternative structural definition has been proposed by Chothia *et al.*, *J.*

Mol. Biol. 196:901 (1987); *Nature* 342:878 (1989); and *J. Mol. Biol.* 186:651 (1989) (hereinafter collectively referred to as "Chothia *et al.*" and incorporated by reference in their entirety for all purposes).

5 A. A_β Antibodies

Therapeutic agents of the invention include antibodies that specifically bind to A_β or to other components of the amyloid plaque. Preferred antibodies are monoclonal antibodies. Some such antibodies bind specifically to the aggregated form of A_β without binding to the soluble form. Some bind specifically to the soluble form without binding to the aggregated form. Some bind to both aggregated and soluble forms. Antibodies used in therapeutic methods preferably have an intact constant region or at least sufficient of the constant region to interact with an Fc receptor. Preferred antibodies are those efficacious at stimulating Fc-mediated phagocytosis of A_β in plaques. Human isotype IgG1 is preferred because of it having highest affinity of 10 human isotypes for the FcRI receptor on phagocytic cells (*e.g.*, on brain resident macrophages or microglial cells). Human IgG1 is the equivalent of murine IgG2a, the latter thus suitable for testing *in vivo* efficacy in animal (*e.g.*, mouse) models of Alzheimer's. Bispecific Fab fragments can also be used, in which one arm of the antibody has specificity for A_β, and the other for an Fc receptor. Preferred antibodies bind to A_β with a binding affinity greater than (or equal to) about 10⁶, 10⁷, 10⁸, 10⁹, or 20 10¹⁰ M⁻¹ (including affinities intermediate of these values).

Monoclonal antibodies bind to a specific epitope within A_β that can be a conformational or nonconformational epitope. Prophylactic and therapeutic efficacy of antibodies can be tested using the transgenic animal model procedures described in the Examples. Preferred monoclonal antibodies bind to an epitope within residues 1-10 of A_β (with the first N terminal residue of natural A_β designated 1), more preferably to an epitope within residues 3-7 of A_β. In some methods, multiple monoclonal antibodies having binding specificities to different epitopes are used, for example, an antibody specific for an epitope within residues 3-7 of A_β can be co-administered with an antibody specific for an epitope outside of residues 3-7 of A_β. Such antibodies can be administered sequentially or simultaneously. Antibodies to amyloid components other than A_β can also be used (*e.g.*, administered or co-administered).

Epitope specificity of an antibody can be determined, for example, by forming a phage display library in which different members display different subsequences of A β . The phage display library is then selected for members specifically binding to an antibody under test. A family of sequences is isolated. Typically, such a 5 family contains a common core sequence, and varying lengths of flanking sequences in different members. The shortest core sequence showing specific binding to the antibody defines the epitope bound by the antibody. Antibodies can also be tested for epitope specificity in a competition assay with an antibody whose epitope specificity has already been determined. For example, antibodies that compete with the 12A11 antibody for 10 binding to A β bind to the same or similar epitope as 12A11, *i.e.*, within residues A β 3-7. Screening antibodies for epitope specificity is a useful predictor of therapeutic efficacy. For example, an antibody determined to bind to an epitope within residues 1-7 of A β is likely to be effective in preventing and treating Alzheimer's disease according to the methodologies of the present invention.

15 Antibodies that specifically bind to a preferred segment of A β without binding to other regions of A β have a number of advantages relative to monoclonal antibodies binding to other regions or polyclonal sera to intact A β . First, for equal mass dosages, dosages of antibodies that specifically bind to preferred segments contain a higher molar dosage of antibodies effective in clearing amyloid plaques. Second, 20 antibodies specifically binding to preferred segments can induce a clearing response against amyloid deposits without inducing a clearing response against intact APP polypeptide, thereby reducing the potential side effects.

1. Production of Nonhuman Antibodies

25 The present invention features non-human antibodies, for example, antibodies having specificity for the preferred A β epitopes of the invention. Such antibodies can be used in formulating various therapeutic compositions of the invention or, preferably, provide complementarity determining regions for the production of humanized or chimeric antibodies (described in detail below). The production of non- 30 human monoclonal antibodies, *e.g.*, murine, guinea pig, primate, rabbit or rat, can be accomplished by, for example, immunizing the animal with A β . A longer polypeptide comprising A β or an immunogenic fragment of A β or anti-idiotypic antibodies to an

antibody to A β can also be used. See Harlow & Lane, *supra*, incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptide synthesis or by recombinant expression. Optionally, the immunogen can be administered fused or otherwise complexed with a carrier protein, as described below.

5 Optionally, the immunogen can be administered with an adjuvant. The term "adjuvant" refers to a compound that when administered in conjunction with an antigen augments the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells,

10 and stimulation of macrophages. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals.

Rabbits or guinea pigs are typically used for making polyclonal antibodies. Exemplary preparation of polyclonal antibodies, e.g., for passive protection, 15 can be performed as follows. 125 non-transgenic mice are immunized with 100 μ g A β 1-42, plus CFA/IFA adjuvant, and euthanized at 4-5 months. Blood is collected from immunized mice. IgG is separated from other blood components. Antibody specific for the immunogen may be partially purified by affinity chromatography. An average of about 0.5-1 mg of immunogen-specific antibody is obtained per mouse, giving a total of 20 60-120 mg.

Mice are typically used for making monoclonal antibodies. Monoclonals can be prepared against a fragment by injecting the fragment or longer form of A β into a mouse, preparing hybridomas and screening the hybridomas for an antibody that specifically binds to A β . Optionally, antibodies are screened for binding to a specific 25 region or desired fragment of A β without binding to other nonoverlapping fragments of A β . The latter screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of an A β peptide and determining which deletion mutants bind to the antibody. Binding can be assessed, for example, by Western blot or ELISA. The smallest fragment to show specific binding to the antibody defines the 30 epitope of the antibody. Alternatively, epitope specificity can be determined by a competition assay in which a test and reference antibody compete for binding to A β . If the test and reference antibodies compete, then they bind to the same epitope or epitopes sufficiently proximal such that binding of one antibody interferes with binding of the

other. The preferred isotype for such antibodies is mouse isotype IgG2a or equivalent isotype in other species. Mouse isotype IgG2a is the equivalent of human isotype IgG1 (*e.g.*, human IgG1).

5 2. Chimeric and Humanized Antibodies

The present invention also features chimeric and/or humanized antibodies (*i.e.*, chimeric and/or humanized immunoglobulins) specific for beta amyloid peptide. Chimeric and/or humanized antibodies have the same or similar binding specificity and affinity as a mouse or other nonhuman antibody that provides the starting material for
10 construction of a chimeric or humanized antibody.

a. Production of Chimeric Antibodies

The term “chimeric antibody” refers to an antibody whose light and heavy chain genes have been constructed, typically by genetic engineering, from
15 immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as IgG1 and IgG4. Human isotype IgG1 is preferred. A typical chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a
20 human antibody.

b. Production of Humanized Antibodies

The term “humanized antibody” refers to an antibody comprising at least one chain comprising variable region framework residues substantially from a human
25 antibody chain (referred to as the acceptor immunoglobulin or antibody) and at least one complementarity determining region substantially from a mouse antibody, (referred to as the donor immunoglobulin or antibody). See, Queen *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989), US 5,530,101, US 5,585,089, US 5,693,761, US 5,693,762, Selick *et al.*, WO 90/07861, and Winter, US 5,225,539 (incorporated by reference in
30 their entirety for all purposes). The constant region(s), if present, are also substantially or entirely from a human immunoglobulin.

The substitution of mouse CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework adopts the same or similar conformation to the mouse variable framework from which the CDRs originated. This is achieved by 5 obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring 10 human antibodies or can be consensus sequences of several human antibodies. See Kettleborough *et al.*, *Protein Engineering* 4:773 (1991); Kolbinger *et al.*, *Protein Engineering* 6:971 (1993) and Carter *et al.*, WO 92/22653.

Having identified the complementarity determining regions of the murine donor immunoglobulin and appropriate human acceptor immunoglobulins, the next step 15 is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune 20 response can be performed to monitor a HAMA response in a particular patient or during clinical trials. Patients administered humanized antibodies can be given an immunogenicity assessment at the beginning and throughout the administration of said therapy. The HAMA response is measured, for example, by detecting antibodies to the humanized therapeutic reagent, in serum samples from the patient using a method 25 known to one in the art, including surface plasmon resonance technology (BIACORE) and/or solid-phase ELISA analysis.

Certain amino acids from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. The unnatural juxtaposition of murine CDR regions with 30 human variable framework region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, lead to loss of binding affinity.

The selection of amino acid residues for substitution is determined, in part, by computer modeling. Computer hardware and software are described herein for producing three-dimensional images of immunoglobulin molecules. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 5 70%, 80%, 90% sequence identity or more are selected for modeling. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying 10 that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

The selection of amino acid residues for substitution can also be determined, in part, by examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of 15 particular amino acids. For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid should usually be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

- 25 (1) noncovalently binds antigen directly,
- (2) is adjacent to a CDR region,
- (3) otherwise interacts with a CDR region (*e.g.*, is within about 3-6 Å of a CDR region as determined by computer modeling), or
- (4) participates in the VL-VH interface.

30 Residues which “noncovalently bind antigen directly” include amino acids in positions in framework regions which have a good probability of directly interacting with amino acids on the antigen according to established chemical forces, for

example, by hydrogen bonding, Van der Waals forces, hydrophobic interactions, and the like.

- CDR and framework regions are as defined by Kabat *et al.* or Chothia *et al., supra*. When framework residues, as defined by Kabat *et al., supra*, constitute structural loop residues as defined by Chothia *et al., supra*, the amino acids present in the mouse antibody may be selected for substitution into the humanized antibody. Residues which are “adjacent to a CDR region” include amino acid residues in positions immediately adjacent to one or more of the CDRs in the primary sequence of the humanized immunoglobulin chain, for example, in positions immediately adjacent to a CDR as defined by Kabat, or a CDR as defined by Chothia (See e.g., Chothia and Lesk JMB 196:901 (1987)). These amino acids are particularly likely to interact with the amino acids in the CDRs and, if chosen from the acceptor, to distort the donor CDRs and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit *et al.*, Science, 233:747 (1986), which is incorporated herein by reference) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

- Residues that “otherwise interact with a CDR region” include those that are determined by secondary structural analysis to be in a spatial orientation sufficient to affect a CDR region. In one embodiment, residues that “otherwise interact with a CDR region” are identified by analyzing a three-dimensional model of the donor immunoglobulin (e.g., a computer-generated model). A three-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDRs are close to the CDRs and have a good probability of interacting with amino acids in the CDRs by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor immunoglobulin amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will generally have a side chain atom within about 3 angstrom units (\AA) of some atom in the CDRs and must contain an atom that could interact with the CDR atoms according to established chemical forces, such as those listed above.

- In the case of atoms that may form a hydrogen bond, the 3 \AA is measured between their nuclei, but for atoms that do not form a bond, the 3 \AA is measured between their Van der Waals surfaces. Hence, in the latter case, the nuclei must be within about 6 \AA (3 \AA plus the sum of the Van der Waals radii) for the atoms to be considered capable

of interacting. In many cases the nuclei will be from 4 or 5 to 6 Å apart. In determining whether an amino acid can interact with the CDRs, it is preferred not to consider the last 8 amino acids of heavy chain CDR 2 as part of the CDRs, because from the viewpoint of structure, these 8 amino acids behave more as part of the framework.

5 Amino acids that are capable of interacting with amino acids in the CDRs, may be identified in yet another way. The solvent accessible surface area of each framework amino acid is calculated in two ways: (1) in the intact antibody, and (2) in a hypothetical molecule consisting of the antibody with its CDRs removed. A significant difference between these numbers of about 10 square angstroms or more shows that
10 access of the framework amino acid to solvent is at least partly blocked by the CDRs, and therefore that the amino acid is making contact with the CDRs. Solvent accessible surface area of an amino acid may be calculated based on a three-dimensional model of an antibody, using algorithms known in the art (e.g., Connolly, *J. Appl. Cryst.* 16:548 (1983) and Lee and Richards, *J. Mol. Biol.* 55:379 (1971), both of which are
15 incorporated herein by reference). Framework amino acids may also occasionally interact with the CDRs indirectly, by affecting the conformation of another framework amino acid that in turn contacts the CDRs.

The amino acids at several positions in the framework are known to be important for determining CDR confirmation (e.g., capable of interacting with the
20 CDRs) in many antibodies (Chothia and Lesk, *supra*, Chothia *et al.*, *supra* and Tramontano *et al.*, *J. Mol. Biol.* 215:175 (1990), all of which are incorporated herein by reference). These authors identified conserved framework residues important for CDR conformation by analysis of the structures of several known antibodies. The antibodies analyzed fell into a limited number of structural or "canonical" classes based on the
25 conformation of the CDRs. Conserved framework residues within members of a canonical class are referred to as "canonical" residues. Canonical residues include residues 2, 25, 29, 30, 33, 48, 64, 71, 90, 94 and 95 of the light chain and residues 24, 26, 29, 34, 54, 55, 71 and 94 of the heavy chain. Additional residues (e.g., CDR structure-determining residues) can be identified according to the methodology of
30 Martin and Thornton (1996) *J. Mol. Biol.* 263:800. Notably, the amino acids at positions 2, 48, 64 and 71 of the light chain and 26-30, 71 and 94 of the heavy chain (numbering according to Kabat) are known to be capable of interacting with the CDRs in many antibodies. The amino acids at positions 35 in the light chain and 93 and 103 in the

heavy chain are also likely to interact with the CDRs. Additional residues which may effect conformation of the CDRs can be identified according to the methodology of Foote and Winter (1992) *J. Mol. Biol.* 224:487. Such residues are termed "vernier" residues and are those residues in the framework region closely underlying (*i.e.*, forming a "platform" under) the CDRs. At all these numbered positions, choice of the donor amino acid rather than the acceptor amino acid (when they differ) to be in the humanized immunoglobulin is preferred. On the other hand, certain residues capable of interacting with the CDR region, such as the first 5 amino acids of the light chain, may sometimes be chosen from the acceptor immunoglobulin without loss of affinity in the humanized immunoglobulin.

10 Residues which "participate in the VL-VH interface" or "packing residues" include those residues at the interface between VL and VH as defined, for example, by Novotny and Haber, *Proc. Natl. Acad. Sci. USA*, 82:4592-66 (1985) or Chothia *et al*, *supra*. Generally, unusual packing residues should be retained in the 15 humanized antibody if they differ from those in the human frameworks.

In general, one or more of the amino acids fulfilling the above criteria can be substituted. In some embodiments, all or most of the amino acids fulfilling the above criteria are substituted. Occasionally, there is some ambiguity about whether a particular amino acid meets the above criteria, and alternative variant immunoglobulins 20 are produced, one of which has that particular substitution, the other of which does not. Alternative variant immunoglobulins so produced can be tested in any of the assays described herein for the desired activity, and the preferred immunoglobulin selected.

Usually the CDR regions in humanized antibodies are substantially identical, and more usually, identical to the corresponding CDR regions of the donor antibody. 25 However, in certain embodiments, it may be desirable to modify one or more CDR regions to modify the antigen binding specificity of the antibody and/or reduce the immunogenicity of the antibody. Typically, one or more residues of a CDR are altered to modify binding to achieve a more favored on-rate of binding, a more favored off-rate of binding, or both, such that an idealized binding constant is achieved. Using this 30 strategy, an antibody having ultra high binding affinity of, for example, 10^{10} M^{-1} or more, can be achieved. Briefly, the donor CDR sequence is referred to as a base sequence from which one or more residues are then altered. Affinity maturation techniques, as described herein, can be used to alter the CDR region(s) followed by

screening of the resultant binding molecules for the desired change in binding. The method may also be used to alter the donor CDR, typically a mouse CDR, to be less immunogenic such that a potential human anti-mouse antibody (HAMA) response is minimized or avoided. Accordingly, as CDR(s) are altered, changes in binding affinity 5 as well as immunogenicity can be monitored and scored such that an antibody optimized for the best combined binding and low immunogenicity are achieved (see, e.g., U.S. Pat. No. 6,656,467 and U.S. Pat. Pub. US20020164326A1).

In another approach, the CDR regions of the antibody are analyzed to determine the contributions of each individual CDR to antibody binding and/or immunogenicity by 10 systemically replacing each of the donor CDRs with a human counterpart. The resultant panel of humanized antibodies is then scored for antigen affinity and potential immunogenicity of each CDR. In this way, the two clinically important properties of a candidate binding molecule, i.e., antigen binding and low immunogenicity, are determined. If patient sera against a corresponding murine or CDR-grafted (humanized) 15 form of the antibody is available, then the entire panel of antibodies representing the systematic human CDR exchanges can be screened to determine the patients anti-idiotypic response against each donor CDR (for technical details, see, e.g., Iwashi *et al.*, *Mol. Immunol.* 36:1079-91 (1999). Such an approach allows for identifying essential donor CDR regions from non-essential donor CDRs. Nonessential donor CDR regions 20 may then be exchanged with a human counterpart CDR. Where an essential CDR region cannot be exchanged without unacceptable loss of function, identification of the specificity-determining residues (SDRs) of the CDR is performed by, for example, site-directed mutagenesis. In this way, the CDR can then be reengineered to retain only the SDRs and be human and/or minimally immunogenic at the remaining amino acid 25 positions throughout the CDR. Such an approach, where only a portion of the donor CDR is grafted, is also referred to as abbreviated CDR-grafting (for technical details on the foregoing techniques, see, e.g., Tamura *et al.*, *J. of Immunology* 164(3):1432-41. (2000); Gonzales *et al.*, *Mol. Immunol* 40:337-349 (2003); Kashmiri *et al.*, *Crit. Rev. Oncol. Hematol.* 38:3-16 (2001); and De Pascalis *et al.*, *J. of Immunology* 169(6):3076-3084. (2002).

Moreover, it is sometimes possible to make one or more conservative amino acid substitutions of CDR residues without appreciably affecting the binding affinity of the resulting humanized immunoglobulin. By conservative substitutions are

intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr.

Additional candidates for substitution are acceptor human framework amino acids that are unusual or “rare” for a human immunoglobulin at that position.

- 5 These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins. For example, substitution may be desirable when the amino acid in a human framework region of the acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position
- 10 in human immunoglobulin sequences; or when the amino acid in the acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is also rare, relative to other human sequences. Whether a residue is rare for acceptor human framework sequences should also be considered when selecting residues for backmutation based on contribution to CDR conformation. For example, if
- 15 backmutation results in substitution of a residue that is rare for acceptor human framework sequences, a humanized antibody may be tested with and without for activity. If the backmutation is not necessary for activity, it may be eliminated to reduce immunogenicity concerns. For example, backmutation at the following residues may introduce a residue that is rare in acceptor human framework sequences; uk = v2(2.0%),
- 20 L3 (0.4%), T7 (1.8%), Q18 (0.2%), L83 (1.2%), I85 (2.9%), A100 (0.3%) and L106 (1.1%); and vh = T3 (2.0%), K5 (1.8%), I11 (0.2%), S23 (1.5%), F24 (1.5%), S41 (2.3%), K71 (2.4%), R75 (1.4%), I82 (1.4%), D83 (2.2%) and L109 (0.8%). These criteria help ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual human acceptor amino acid
- 25 with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

The term “rare”, as used herein, indicates an amino acid occurring at that position in less than about 20%, preferably less than about 10%, more preferably less than about 5%, even more preferably less than about 3%, even more preferably less than about 2% and even more preferably less than about 1% of sequences in a representative sample of sequences, and the term “common”, as used herein, indicates an amino acid occurring in more than about 25% but usually more than about 50% of sequences in a representative sample. For example, when deciding whether an amino acid in a human

acceptor sequence is "rare" or "common", it will often be preferable to consider only human variable region sequences and when deciding whether a mouse amino acid is "rare" or "common", only mouse variable region sequences. Moreover, all human light and heavy chain variable region sequences are respectively grouped into "subgroups" of sequences that are especially homologous to each other and have the same amino acids at certain critical positions (*Kabat et al., supra*). When deciding whether an amino acid in a human acceptor sequence is "rare" or "common" among human sequences, it will often be preferable to consider only those human sequences in the same subgroup as the acceptor sequence.

10 Additional candidates for substitution are acceptor human framework amino acids that would be identified as part of a CDR region under the alternative definition proposed by Chothia *et al., supra*. Additional candidates for substitution are acceptor human framework amino acids that would be identified as part of a CDR region under the AbM and/or contact definitions.

15 Additional candidates for substitution are acceptor framework residues that correspond to a rare or unusual donor framework residue. Rare or unusual donor framework residues are those that are rare or unusual (as defined herein) for murine antibodies at that position. For murine antibodies, the subgroup can be determined according to Kabat and residue positions identified which differ from the consensus.

20 These donor specific differences may point to somatic mutations in the murine sequence which enhance activity. Unusual residues that are predicted to affect binding (*e.g.*, packing canonical and/or vernier residues) are retained, whereas residues predicted to be unimportant for binding can be substituted. Rare residues within the 12A11 UK sequence include I85 (3.6%). Rare residues within the 12A11 vh sequence include T3 (1.0%), I11 (1.7%), L12 (1.7%), S41 (2.8%), D83 (1.8%) and A85 (1.8%).

25 Additional candidates for substitution are non-germline residues occurring in an acceptor framework region. For example, when an acceptor antibody chain (*i.e.*, a human antibody chain sharing significant sequence identity with the donor antibody chain) is aligned to a germline antibody chain (likewise sharing significant sequence identity with the donor chain), residues not matching between acceptor chain framework and the germline chain framework can be substituted with corresponding residues from the germline sequence.

Other than the specific amino acid substitutions discussed above, the framework regions of humanized immunoglobulins are usually substantially identical, and more usually, identical to the framework regions of the human antibodies from which they were derived. Of course, many of the amino acids in the framework region make little or no direct contribution to the specificity or affinity of an antibody. Thus, many individual conservative substitutions of framework residues can be tolerated without appreciable change of the specificity or affinity of the resulting humanized immunoglobulin. Thus, in one embodiment the variable framework region of the humanized immunoglobulin shares at least 85% sequence identity to a human variable framework region sequence or consensus of such sequences. In another embodiment, the variable framework region of the humanized immunoglobulin shares at least 90%, preferably 95%, more preferably 96%, 97%, 98% or 99% sequence identity to a human variable framework region sequence or consensus of such sequences. In general, however, such substitutions are undesirable.

In exemplary embodiments, the humanized antibodies of the invention exhibit a specific binding affinity for antigen of at least 10^7 , 10^8 , 10^9 or 10^{10} M⁻¹. In other embodiments, the antibodies of the invention can have binding affinities of at least 10^{10} , 10^{11} or 10^{12} M⁻¹. Usually the upper limit of binding affinity of the humanized antibodies for antigen is within a factor of three, four or five of that of the donor immunoglobulin. Often the lower limit of binding affinity is also within a factor of three, four or five of that of donor immunoglobulin. Alternatively, the binding affinity can be compared to that of a humanized antibody having no substitutions (*e.g.*, an antibody having donor CDRs and acceptor FRs, but no FR substitutions). In such instances, the binding of the optimized antibody (with substitutions) is preferably at least two- to three-fold greater, or three- to four-fold greater, than that of the unsubstituted antibody. For making comparisons, activity of the various antibodies can be determined, for example, by BIACORE (*i.e.*, surface plasmon resonance using unlabelled reagents) or competitive binding assays.

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c. Production of Humanized 12A11 Antibodies

A preferred embodiment of the present invention features a humanized antibody to the N-terminus of A β , in particular, for use in the therapeutic and/or diagnostic methodologies described herein. A particularly preferred starting material for

production of humanized antibodies is the monoclonal antibody 12A11. 12A11 is specific for the N-terminus of A β and has been shown to (1) have a high avidity for aggregated A β 1-42, (2) have the ability to capture soluble A β , and (3) mediate phagocytosis (*e.g.*, induce phagocytosis) of amyloid plaque (see Example I). The *in vivo* efficacy of the 12A11 antibody is described in Example II. The cloning and sequencing of cDNA encoding the 12A11 antibody heavy and light chain variable regions is described in Example III.

Suitable human acceptor antibody sequences can be identified by computer comparisons of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies. The comparison is performed separately for heavy and light chains but the principles are similar for each. In particular, variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine VL and VH framework regions are identified by query of, for example, the Kabat Database or the IgG Protein Sequence Database using NCBI IgG BLAST (publicly accessible through the National Institutes of Health NCBI internet server) with the respective murine framework sequences. In one embodiment, acceptor sequences sharing greater than 50% sequence identity with murine donor sequences, *e.g.*, donor framework (FR) sequences, are selected. Preferably, acceptor antibody sequences sharing 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% sequence identity or more are selected.

A computer comparison of 12A11 revealed that the 12A11 light chain (mouse subgroup II) shows the greatest sequence identity to human light chains of subtype kappa II, and that the 12A11 heavy chain (mouse subgroup Ib) shows greatest sequence identity to human heavy chains of subtype II, as defined by Kabat *et al.*, *supra*. Light and heavy human framework regions can be derived from human antibodies of these subtypes, or from consensus sequences of such subtypes. In a first humanization effort, light chain variable framework regions were derived from human subgroup II antibodies. Based on previous experiments designed to achieve high levels of expression of humanized antibodies having heavy chain variable framework regions derived from human subgroup II antibodies, it had been discovered that expression levels of such antibodies were sometimes low. Accordingly, based on the reasoning described in Saldanha *et al.* (1999) *Mol Immunol.* 36:709-19, framework regions from human subgroup III antibodies were chosen rather than human subgroup II.

A human subgroup II antibody K64(AIMS4) (accession no. BAC01733) was identified from the NCBI non-redundant database having significant sequence identity within the light chain variable regions of 12A11. A human subgroup III antibody M72 (accession no. AAA69734) was identified from the NCBI non-redundant database having significant sequence identity within the heavy chain variable regions of 12A11 (see also Schroeder and Wang (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87: 6146-6150.

Alternative light chain acceptor sequences include, for example, PDB Accession No. 1KFA (gi24158782), PDB Accession No. 1KFA (gi24158784), EMBL Accession No. CAE75574.1 (gi38522587), EMBL Accession No. CAE75575.1 (gi38522590), EMBL Accession No. CAE84952.1 (gi39838891), DJB Accession No. BAC01734.1 (gi21669419), DJB Accession No. BAC01730.1 (gi21669411), PIR Accession No. S40312 (gi481978), EMBL Accession No. CAA51090.1 (gi3980118), GenBank Accession No. AAH63599.1 (gi39794308), PIR Accession No. S22902 (gi106540), PIR Accession No. S42611 (gi631215), EMBL Accession No. CAA38072.1 (gi433890), GenBank Accession No. AAD00856.1 (gi4100384), EMBL Accession No. CAA39072.1 (gi34000), PIR Accession No. S23230 (gi284256), DBJ Accession No. BAC01599.1 (gi21669149), DBJ Accession No. BAC01729.1 (gi21669409), DBJ Accession No. BAC01562.1 (gi21669075), EMBL Accession No. CAA85590.1 (gi587338), GenBank Accession No. AAQ99243.1 (gi37694665), GenBank Accession No. AAK94811.1 (gi18025604), EMBL Accession No. CAB51297.1 (gi5578794), DBJ Accession No. BAC01740.1 (gi21669431), and DBJ Accession No. BAC01733.1 (gi21669417). Alternative heavy chain acceptor sequences include, for example, GenBank Accession No. AAB35009.1 (gi1041885), DBJ Accession No. BAC01904.1 (gi21669789), GenBank Accession No. AAD53816.1 (gi5834100), GenBank Accession No. AAS86081.1 (gi46254223), DBJ Accession No. BAC01462.1 (gi21668870), GenBank Accession No. AAC18191.1 (gi3170773), DBJ Accession No. BAC02266.1 (gi21670513), GenBank Accession No. AAD56254.1 (gi5921589), GenBank Accession No. AAD53807.1 (gi5834082), DBJ Accession No. BAC02260.1 (gi21670501), GenBank Accession No. AAC18166.1 (gi3170723), EMBL Accession No. CAA49495.1 (gi33085), PIR Accession No. S31513 (gi345903), GenBank Accession No. AAS86079.1 (gi46254219), DBJ Accession No. BAC01917.1 (gi21669815), DBJ Accession No. BAC01912.1 (gi21669805), GenBank Accession No. AAC18283.1

(gi3170961), DBJ Accession No. BAC01903 (gi21669787), DBJ Accession NO. BAC01887.1 (gi21669755), DBJ Accession No. BAC02259.1 (gi21370499), DBJ Accession No. BAC01913.1 (gi21669807), DBJ Accession No. BAC01910.1 (gi21669801), DJB Accession No. BAC02267.1 (gi21670515), GenBank Accession No. AAC18306.1 (gi3171011), GenBank Accession No. AAD53817.1 (gi5834102), PIR Accession No. E36005 (gi106423), EMBL CAB37129.1 (gi4456494) and GenBank AAA68892.1 (gi186190).

In exemplary embodiments, humanized antibodies of the invention include 12A11 CDRs and FRs from an acceptor sequence listed *supra*. Residues within the framework regions important for CDR conformation and/or activity as described herein are selected for backmutation (if differing between donor and acceptor sequences).

Residues are next selected for substitution, as follows. When an amino acid differs between a 12A11 variable framework region and an equivalent human variable framework region, the human framework amino acid should usually be substituted by the equivalent mouse amino acid if it is reasonably expected that the amino acid:

- (1) noncovalently binds antigen directly,
- (2) is adjacent to a CDR region, is part of a CDR region under the alternative definition proposed by Chothia *et al.*, *supra*, or otherwise interacts with a CDR region (*e.g.*, is within about 3A of a CDR region), or
- (3) participates in the VL-VH interface.

Structural analysis of the 12A11 antibody heavy and light chain variable regions, and humanization of the 12A11 antibody is described in Example V. Briefly, three-dimensional models for the solved murine antibody structures 1KTR for the light chain and 1JRH and 1ETZ for the heavy chain were studied. Alternative three-dimensional models which can be studied for identification of residues, important for CDR confirmation (*e.g.*, vernier residues), include PDB Accession No. 2JEL (gi3212688), PDB Accession No. 1TET (gi494639), PDB Accession No. IJP5 (gi16975307), PDB Accession No. 1CBV (gi493917), PDB Accession No. 2PCP (gi4388943), PDB Accession No. 1I9I (gi2050118), PDB Accession No. 1CLZ (gi1827926), PDB Accession No. 1FL6 (gi17942615) and PDB Accession No. 1KEL (gi1942968) for the light chain and PDB 1GGI (gi442938), PDB Accession No. 1GGB

(gi442934), PDB Accession No. 1N5Y (gi28373913), PDB Accession No. 2HMI (gi3891821), PDB Accession No. 1FDL (gi229915), PDB Accession No. 1KIP (gi1942788), PDB Accession No. 1KIQ (gi1942791) and PDB Accession No. 1VFA (gi576325) for the heavy chain.

5 Three-dimensional structural information for the antibodies described herein is publicly available, for example, from the Research Collaboratory for Structural Bioinformatics' Protein Data Bank (PDB). The PDB is freely accessible *via* the World Wide Web internet and is described by Berman *et al.* (2000) *Nucleic Acids Research*, 28:235. Study of solved three-dimensional structures allows for the identification of
10 CDR-interacting residues within 12A11. Alternatively, three-dimensional models for the 12A11 VH and VL chains can be generated using computer modeling software. Briefly, a three-dimensional model is generated based on the closest solved murine antibody structures for the heavy and light chains. For this purpose, 1KTR can be used as a template for modeling the 12A11 light chain, and 1ETZ and 1JRH used as templates
15 for modeling the heavy chain. The model can be further refined by a series of energy minimization steps to relieve unfavorable atomic contacts and optimize electrostatic and van der Waals interactions. Additional three-dimensional analysis and/or modeling can be performed using 2JEL (2.5Å) and/or 1TET (2.3Å) for the light chain and 1GGI (2.8Å) for the heavy chain (or other antibodies set forth *supra*) based on the similarity
20 between these solved murine structures and the respective 12A11 chains.

 The computer model of the structure of 12A11 can further serve as a starting point for predicting the three-dimensional structure of an antibody containing the 12A11 complementarity determining regions substituted in human framework structures. Additional models can be constructed representing the structure as further
25 amino acid substitutions are introduced.

 In general, substitution of one, most or all of the amino acids fulfilling the above criteria is desirable. Accordingly, the humanized antibodies of the present invention will usually contain a substitution of a human light chain framework residue with a corresponding 12A11 residue in at least 1, 2, 3 or more of the chosen positions.
30 The humanized antibodies also usually contain a substitution of a human heavy chain framework residue with a corresponding 12A11 residue in at least 1, 2, 3 or more of the chosen positions.

- Occasionally, however, there is some ambiguity about whether a particular amino acid meets the above criteria, and alternative variant immunoglobulins are produced, one of which has that particular substitution, the other of which does not. In instances where substitution with a murine residue would introduce a residue that is rare in human immunoglobulins at a particular position, it may be desirable to test the antibody for activity with or without the particular substitution. If activity (e.g., binding affinity and/or binding specificity) is about the same with or without the substitution, the antibody without substitution may be preferred, as it would be expected to elicit less of a HAMA response, as described herein.
- Other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of more typical human immunoglobulins. Alternatively, amino acids from equivalent positions in the mouse 12A11 can be introduced into the human framework regions when such amino acids are typical of human immunoglobulin at the equivalent positions.

Other candidates for substitution are non-germline residues occurring in a framework region. By performing a computer comparison of 12A11 with known germline sequences, germline sequences with the greatest degree of sequence identity to the heavy or light chain can be identified. Alignment of the framework region and the germline sequence will reveal which residues may be selected for substitution with corresponding germline residues. Residues not matching between a selected light chain acceptor framework and one of these germline sequences could be selected for substitution with the corresponding germline residue.

Rare mouse residues are identified by comparing the donor VL and/or VH sequences with the sequences of other members of the subgroup to which the donor VL and/or VH sequences belong (according to Kabat) and identifying the residue positions which differ from the consensus. These donor specific differences may point to somatic mutations which enhance activity. Unusual or rare residues close to the binding site may possibly contact the antigen, making it desirable to retain the mouse residue. However, if the unusual mouse residue is not important for binding, use of the corresponding acceptor residue is preferred as the mouse residue may create immunogenic neoepitopes in the humanized antibody. In the situation where an unusual

residue in the donor sequence is actually a common residue in the corresponding acceptor sequence, the preferred residue is clearly the acceptor residue.

Table 1A summarizes the sequence analysis of the 12A11 VH and VL regions.

5

Table 1. Summary of 12A11 V region sequence

Chain	VL	VH
Mouse Subgroup	II	Ib
Human Subgroup	II	II
Rare amino acids in mouse vk (% frequency)	I85 (3.6%)	I11 (1.7%)
Chothia canonical class	L1: class 4[16f] L2: class 1[7] L3: class 1[9]	H1: class 3 [7] H2: class 1[16] H3 ¹
Closest mouse MAb solved structure	1KTR ²	1ETZ ³ (2.6Å) and 1JRH ⁴
Homology with Modeling template	94%	83% and 86%
Human Framework seq	K64 (BAC01733) (87% FR, 67% overall)	M72 (AAA69734) (61% FR, 45% overall)
Donornotes	Hu k LC subgroup II CDRs from same canonical Structural group as 12A11	HU HC subgroup III CDRs from same canonical structural group as 12A11

Backmutation Notes	none	A24F, F29L: H1 R71K: Canonical, H2 V37I: Packing T28S, V48L, F67L, N73T, L78V: Vernier
Germline ref for Hu Fr	A19 VL Vk2-28 mRNA: X63397.1 (GI:33774)	AAA69731.1 (GI:567123)

¹ No canonical class but might form a kinked base according to the rules of Shirai *et al.* (1999) *FEBS Lett.* 455:188-197.

² Kaufmann *et al.* (2002) *J Mol Biol.* 318:135-147.

5 ³ Guddat *et al.* (2000) *J Mol Biol.* 302:853-872.

⁴ Sogabe *et al.* (1997) *J Mol Biol.* 273:882-897.

Germline sequences are set forth that can be used in selecting amino acid substitutions.

10

Three-dimensional structural information for antibodies described herein is publicly available, for example, from the Research Collaboratory for Structural Bioinformatics' Protein Data Bank (PDB). The PDB is freely accessible via the World Wide Web internet and is described by Berman *et al.* (2000) *Nucleic Acids Research*,

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p235-242. Germline gene sequences referenced herein are publicly available, for example, from the National Center for Biotechnology Information (NCBI) database of sequences in collections of IgH, Ig kappa and Ig lambda germline V genes (as a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH)). Homology searching of the NCBI "Ig Germline Genes" database is provided by IgG

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In an exemplary embodiment, a humanized antibody of the present invention contains (i) a light chain comprising a variable domain comprising murine 12A11 VL CDRs and a human acceptor framework, the framework having zero, one, two, three, four, five, six, seven, eight, nine or more residues substituted with the corresponding 12A11 residue and (ii) a heavy chain comprising 12A11 VH CDRs and a

human acceptor framework, the framework having at least one, two, three, four, five, six, seven, eight, nine or more residues substituted with the corresponding 12A11 residue, and, optionally, at least one, preferably two or three residues substituted with a corresponding human germline residue.

5 In another exemplary embodiment, a humanized antibody of the present invention contains (i) a light chain comprising a variable domain comprising murine 12A11 VL CDRs and a human acceptor framework, the framework having at least one, two, three, four, five, six, seven, eight, nine or more residues backmutated (*i.e.*, substituted with the corresponding 12A11 residue), wherein the backmutation(s) are at a canonical, packing and/or vernier residues and (ii) a heavy chain comprising 12A11 VH CDRs and a human acceptor framework, the framework having at least one, two, three, four, five, six, seven, eight, nine or more residues bacmutated, wherein the backmutation(s) are at a canonical, packing and/or vernier residues. In certain embodiments, backmutations are only at packing and/or canonical residues or are primarily at canonical and/or packing residues (*e.g.*, only 1 or 2 vernier residues of the vernier residues differing between the donor and acceptor sequence are backmutated).

In other embodiments, humanized antibodies include the fewest number of backmutations possible while retaining a binding affinity comparable to that of the donor antibody (or a chimeric version thereof). To arrive at such versions, various combinations of backmutations can be eliminated and the resulting antibodies tested for efficacy (*e.g.*, binding affinity). For example, backmutations (*e.g.*, 1, 2, 3, or 4 backmutations) at vernier residues can be eliminated or backmutations at combinations of vernier and packing, vernier and canonical or packing and canonical residues can be eliminated.

25 In another embodiment, a humanized antibody of the present invention has structural features, as described herein, and further has at least one (preferably two, three, four or all) of the following activities: (1) binds soluble A β ; (2) binds aggregated A β 1-42 (*e.g.*, as determined by ELISA); (3) captures soluble A β ; (4) binds A β in plaques (*e.g.*, staining of AD and/or PDAPP plaques); (5) binds A β with an affinity no less than two to three fold lower than chimeric 12A11 (*e.g.*, 12A11 having murine variable region sequences and human constant region sequences); (6) mediates phagocytosis of A β (*e.g.*, in an *ex vivo* phagocytosis assay, as described herein); and (7)

crosses the blood-brain barrier (*e.g.*, demonstrates short-term brain localization, for example, in a PDAPP animal model, as described herein).

In another embodiment, a humanized antibody of the present invention has structural features, as described herein, such that it binds A β in a manner or with an affinity sufficient to elicit at least one of the following *in vivo* effects: (1) reduce A β plaque burden; (2) prevent plaque formation; (3) reduce levels of soluble A β ; (4) reduce the neuritic pathology associated with an amyloidogenic disorder; (5) lessen or ameliorate at least one physiological symptom associated with an amyloidogenic disorder; and/or (6) improve cognitive function.

10 In another embodiment, a humanized antibody of the present invention has structural features, as described herein, and specifically binds to an epitope comprising residues 3-7 of A β .

15 In yet another embodiment, a humanized antibody of the present invention has structural features, as described herein, such that it binds to an N-terminal epitope within A β (*e.g.*, binds to an epitope within amino acids 3-7 of A β), and is capable of reducing (1) A β peptide levels; (2) A β plaque burden; and (3) the neuritic burden or neuritic dystrophy associated with an amyloidogenic disorder.

20 The activities described above can be determined utilizing any one of a variety of assays described herein or in the art (*e.g.*, binding assays, phagocytosis assays, etc.). Activities can be assayed either *in vivo* (*e.g.*, using labeled assay components and/or imaging techniques) or *in vitro* (*e.g.*, using samples or specimens derived from a subject). Activities can be assayed either directly or indirectly. In certain preferred embodiments, neurological endpoints (*e.g.*, amyloid burden, neuritic burden, etc) are assayed. Such endpoints can be assayed in living subjects (*e.g.*, in animal models of Alzheimer's disease or in human subjects, for example, undergoing immunotherapy) using non-invasive detection methodologies. Alternatively, such endpoints can be assayed in subjects post mortem. Assaying such endpoints in animal models and/or in human subjects post mortem is useful in assessing the effectiveness of various agents (*e.g.*, humanized antibodies) to be utilized in similar immunotherapeutic applications. In 25 other preferred embodiments, behavioral or neurological parameters can be assessed as indicators of the above neuropathological activities or endpoints.

3. Production of Variable Regions

Having conceptually selected the CDR and framework components of humanized immunoglobulins, a variety of methods are available for producing such immunoglobulins. In general, one or more of the murine complementarity determining regions (CDR) of the heavy and/or light chain of the antibody can be humanized, for example, placed in the context of one or more human framework regions, using primer-based polymerase chain reaction (PCR). Briefly, primers are designed which are capable of annealing to target murine CDR region(s) which also contain sequence which overlaps and can anneal with a human framework region. Accordingly, under appropriate conditions, the primers can amplify a murine CDR from a murine antibody template nucleic acid and add to the amplified template a portion of a human framework sequence. Similarly, primers can be designed which are capable of annealing to a target human framework region(s) where a PCR reaction using these primers results in an amplified human framework region(s). When each amplification product is then denatured, combined, and annealed to the other product, the murine CDR region, having overlapping human framework sequence with the amplified human framework sequence, can be genetically linked. Accordingly, in one or more such reactions, one or more murine CDR regions can be genetically linked to intervening human framework regions.

In some embodiments, the primers may also comprise desirable restriction enzyme recognition sequences to facilitate the genetic engineering of the resultant PCR amplified sequences into a larger genetic segment, for example, a variable light or heavy chain segment, heavy chain, or vector. In addition, the primers used to amplify either the murine CDR regions or human framework regions may have desirable mismatches such that a different codon is introduced into the murine CDR or human framework region. Typical mismatches introduce alterations in the human framework regions that preserve or improve the structural orientation of the murine CDR and thus its binding affinity, as described herein.

It should be understood that the foregoing approach can be used to introduce one, two, or all three murine CDR regions into the context of intervening human framework regions. Methods for amplifying and linking different sequences using primer-based PCR are described in, for example, Sambrook, Fritsch and Maniatis, *Molecular Cloning: Cold Spring Harbor Laboratory Press* (1989); *DNA Cloning*, Vols. 1 and 2, (D.N.

Glover, Ed. 1985); *PCR Handbook Current Protocols in Nucleic Acid Chemistry*, Beaucage, Ed. John Wiley & Sons (1999) (Editor); *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*, John Wiley & Sons (1992).

Because of the degeneracy of the code, a variety of nucleic acid sequences will 5 encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an earlier prepared variant of the desired polynucleotide. Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion and insertion variants of target polypeptide DNA. See Adelman *et al.*, DNA 2:183 (1983). Briefly, 10 the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer, and encodes the selected alteration in the target polypeptide DNA.

15

4. Selection of Constant Regions

The variable segments of antibodies produced as described *supra* (e.g., the heavy and light chain variable regions of chimeric or humanized antibodies) are typically linked to at least a portion of an immunoglobulin constant region (Fc region), 20 typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B cells (see Kabat *et al.*, *supra*, and Liu *et al.*, W087/02671) (each of which is incorporated by reference in its entirety for all purposes). Ordinarily, the antibody will contain both light chain and heavy chain constant regions. The heavy 25 chain constant region usually includes CH1, hinge, CH2, CH3, and CH4 regions. The antibodies described herein include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. When it is desired that the antibody (e.g., humanized antibody) exhibit cytotoxic activity, the constant domain is usually a complement fixing constant domain and the 30 class is typically IgG1. Human isotype IgG1 is preferred. Light chain constant regions can be lambda or kappa. The humanized antibody may comprise sequences from more than one class or isotype. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab' F(ab')2, and

Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

5. Expression of Recombinant Antibodies

Chimeric and humanized antibodies are typically produced by recombinant expression. Nucleic acids encoding light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells (*e.g.*, COS cells). Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies.

These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers (*e.g.*, ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences (see, *e.g.*, Itakura *et al.*, US Patent 4,704,362).

E. coli is one prokaryotic host particularly useful for cloning the polynucleotides (*e.g.*, DNA sequences) of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control

expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, are also useful for expression.

Saccharomyces is a preferred yeast host, with suitable vectors having expression control sequences (e.g., promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (e.g., polynucleotides encoding immunoglobulins or fragments thereof). See Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y. (1987). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting heterologous proteins (e.g., intact immunoglobulins) have been developed in the art, and include CHO cell lines, various Cos cell lines, HeLa cells, preferably, myeloma cell lines, or transformed B-cells or hybridomas. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (Queen *et al.*, *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, cytomegalovirus and the like. See Co *et al.*, *J. Immunol.* 148:1149 (1992).

Alternatively, antibody-coding sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (see, e.g., Deboer *et al.*, US 5,741,957, Rosen, US 5,304,489, and Meade *et al.*, US 5,849,992). Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

Alternatively, antibodies (e.g., humanized antibodies) of the invention can be produced in transgenic plants (e.g., tobacco, maize, soybean and alfalfa). Improved 'plantibody' vectors (Hendy *et al.* (1999) *J. Immunol. Methods* 231:137-146) and

purification strategies coupled with an increase in transformable crop species render such methods a practical and efficient means of producing recombinant immunoglobulins not only for human and animal therapy, but for industrial applications as well (e.g., catalytic antibodies). Moreover, plant produced antibodies have been shown to be safe and 5 effective and avoid the use of animal-derived materials and therefore the risk of contamination with a transmissible spongiform encephalopathy (TSE) agent. Further, the differences in glycosylation patterns of plant and mammalian cell-produced antibodies have little or no effect on antigen binding or specificity. In addition, no evidence of toxicity or HAMA has been observed in patients receiving topical oral application of a 10 plant-derived secretory dimeric IgA antibody (see Larrick *et al.* (1998) *Res. Immunol.* 149:603-608).

Various methods may be used to express recombinant antibodies in transgenic plants. For example, antibody heavy and light chains can be independently cloned into expression vectors (e.g., *Agrobacterium tumefaciens* vectors), followed by the 15 transformation of plant tissue *in vitro* with the recombinant bacterium or direct transformation using, e.g., particles coated with the vector which are then physically introduced into the plant tissue using, e.g., ballistics. Subsequently, whole plants expressing individual chains are reconstituted followed by their sexual cross, ultimately resulting in the production of a fully assembled and functional antibody. Similar protocols 20 have been used to express functional antibodies in tobacco plants (see Hiatt *et al.* (1989) *Nature* 342:76-87). In various embodiments, signal sequences may be utilized to promote the expression, binding and folding of unassembled antibody chains by directing the chains to the appropriate plant environment (e.g., the aqueous environment of the apoplasm or other specific plant tissues including tubers, fruit or seed) (see Fiedler *et al.* 25 (1995) *Bio/Technology* 13:1090-1093). Plant bioreactors can also be used to increase antibody yield and to significantly reduce costs.

The vectors containing the polynucleotide sequences of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type 30 of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (See generally Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press,

2nd ed., 1989) (incorporated by reference in its entirety for all purposes). Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (see generally, Sambrook *et al.*, *supra*). For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

When heavy and light chains are cloned on separate expression vectors, the vectors are co-transfected to obtain expression and assembly of intact immunoglobulins. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, HPLC purification, gel electrophoresis and the like (see generally Scopes, *Protein Purification* (Springer-Verlag, N.Y., (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses.

6. Antibody Fragments

Also contemplated within the scope of the instant invention are antibody fragments. In one embodiment, fragments of non-human, and/or chimeric antibodies are provided. In another embodiment, fragments of humanized antibodies are provided. Typically, these fragments exhibit specific binding to antigen with an affinity of at least 10^7 , and more typically 10^8 or $10^9 M^{-1}$. Humanized antibody fragments include separate heavy chains, light chains, Fab, Fab', F(ab')2, Fabc, and Fv. Fragments are produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

7. Epitope Mapping

Epitope mapping can be performed to determine which antigenic determinant or epitope of A β is recognized by the antibody. In one embodiment, epitope mapping is performed according to Replacement NET (rNET) analysis. The rNET epitope map assay provides information about the contribution of individual residues within the epitope to the overall binding activity of the antibody. rNET analysis

uses synthesized systematic single substituted peptide analogs. Binding of an antibody being tested is determined against native peptide (native antigen) and against 19 alternative "single substituted" peptides, each peptide being substituted at a first position with one of 19 non-native amino acids for that position. A profile is generated reflecting 5 the effect of substitution at that position with the various non-native residues. Profiles are likewise generated at successive positions along the antigenic peptide. The combined profile, or epitope map, (reflecting substitution at each position with all 19 non-native residues) can then be compared to a map similarly generated for a second antibody. Substantially similar or identical maps indicate that antibodies being 10 compared have the same or similar epitope specificity.

8. Testing Antibodies for Therapeutic Efficacy in Animal Models

Groups of 7-9 month old PDAPP mice each are injected with 0.5 mg in PBS of polyclonal anti-A β or specific anti-A β monoclonal antibodies. All antibody 15 preparations are purified to have low endotoxin levels. Monoclonals can be prepared against a fragment by injecting the fragment or longer form of A β into a mouse, preparing hybridomas and screening the hybridomas for an antibody that specifically binds to a desired fragment of A β without binding to other nonoverlapping fragments of A β .

20 Mice are injected intraperitoneally as needed over a 4 month period to maintain a circulating antibody concentration measured by ELISA titer of greater than 1/1000 defined by ELISA to A β 42 or other immunogen. Titers are monitored and mice are euthanized at the end of 6 months of injections. Histochemistry, A β levels and toxicology are performed post mortem. Ten mice are used per group.

25

9. Screening Antibodies for Clearing Activity

The invention also provides methods of screening an antibody for activity in clearing an amyloid deposit or any other antigen, or associated biological entity, for which clearing activity is desired. To screen for activity against an amyloid deposit, a 30 tissue sample from a brain of a patient with Alzheimer's disease or an animal model having characteristic Alzheimer's pathology is contacted with phagocytic cells bearing an Fc receptor, such as microglial cells, and the antibody under test in a medium *in vitro*. The phagocytic cells can be a primary culture or a cell line, and can be of murine (e.g.,

BV-2 or C8-B4 cells) or human origin (e.g., THP-1 cells). In some methods, the components are combined on a microscope slide to facilitate microscopic monitoring. In some methods, multiple reactions are performed in parallel in the wells of a microtiter dish. In such a format, a separate miniature microscope slide can be mounted in the 5 separate wells, or a nonmicroscopic detection format, such as ELISA detection of A β can be used. Preferably, a series of measurements is made of the amount of amyloid deposit in the *in vitro* reaction mixture, starting from a baseline value before the reaction has proceeded, and one or more test values during the reaction. The antigen can be detected by staining, for example, with a fluorescently labeled antibody to A β or other 10 component of amyloid plaques. The antibody used for staining may or may not be the same as the antibody being tested for clearing activity. A reduction relative to baseline during the reaction of the amyloid deposits indicates that the antibody under test has clearing activity. Such antibodies are likely to be useful in preventing or treating Alzheimer's and other amyloidogenic diseases. Particularly useful antibodies for 15 preventing or treating Alzheimer's and other amyloidogenic diseases include those capable of clearing both compact and diffuse amyloid plaques, for example, the 12A11 antibody of the instant invention, or chimeric or humanized versions thereof.

Analogous methods can be used to screen antibodies for activity in clearing other types of biological entities. The assay can be used to detect clearing 20 activity against virtually any kind of biological entity. Typically, the biological entity has some role in human or animal disease. The biological entity can be provided as a tissue sample or in isolated form. If provided as a tissue sample, the tissue sample is preferably unfixed to allow ready access to components of the tissue sample and to avoid perturbing the conformation of the components incidental to fixing. Examples of 25 tissue samples that can be tested in this assay include cancerous tissue, precancerous tissue, tissue containing benign growths such as warts or moles, tissue infected with pathogenic microorganisms, tissue infiltrated with inflammatory cells, tissue bearing pathological matrices between cells (e.g., fibrinous pericarditis), tissue bearing aberrant antigens, and scar tissue. Examples of isolated biological entities that can be used 30 include A β , viral antigens or viruses, proteoglycans, antigens of other pathogenic microorganisms, tumor antigens, and adhesion molecules. Such antigens can be obtained from natural sources, recombinant expression or chemical synthesis, among other means. The tissue sample or isolated biological entity is contacted with

phagocytic cells bearing Fc receptors, such as monocytes or microglial cells, and an antibody to be tested in a medium. The antibody can be directed to the biological entity under test or to an antigen associated with the entity. In the latter situation, the object is to test whether the biological entity is phagocytosed with the antigen. Usually, although 5 not necessarily, the antibody and biological entity (sometimes with an associated antigen), are contacted with each other before adding the phagocytic cells. The concentration of the biological entity and/or the associated antigen remaining in the medium, if present, is then monitored. A reduction in the amount or concentration of antigen or the associated biological entity in the medium indicates the antibody has a 10 clearing response against the antigen and/or associated biological entity in conjunction with the phagocytic cells.

10. Chimeric / Humanized Antibodies Having Altered Effector Function

For the above-described antibodies of the invention comprising a constant 15 region (Fc region), it may also be desirable to alter the effector function of the molecule. Generally, the effector function of an antibody resides in the constant or Fc region of the molecule which can mediate binding to various effector molecules, e.g., complement proteins or Fc receptors. The binding of complement to the Fc region is important, for example, in the opsonization and lysis of cell pathogens and the activation of 20 inflammatory responses. The binding of antibody to Fc receptors, for example, on the surface of effector cells can trigger a number of important and diverse biological responses including, for example, engulfment and destruction of antibody-coated pathogens or particles, clearance of immune complexes, lysis of antibody-coated target 25 cells by killer cells (i.e., antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer of antibodies, and control of immunoglobulin production.

Accordingly, depending on a particular therapeutic or diagnostic application, the above-mentioned immune functions, or only selected immune functions, may be desirable. By altering the Fc region of the antibody, various aspects of the 30 effector function of the molecule, including enhancing or suppressing various reactions of the immune system, with beneficial effects in diagnosis and therapy, are achieved.

The antibodies of the invention can be produced which react only with certain types of Fc receptors, for example, the antibodies of the invention can be modified to bind to only certain Fc receptors, or if desired, lack Fc receptor binding entirely, by deletion or alteration of the Fc receptor binding site located in the Fc region of the antibody. Other desirable alterations of the Fc region of an antibody of the invention are cataloged below. Typically the Kabat numbering system is used to indicate which amino acid residue(s) of the Fc region (*e.g.*, of an IgG antibody) are altered (*e.g.*, by amino acid substitution) in order to achieve a desired change in effector function. The numbering system is also employed to compare antibodies across species such that a desired effector function observed in, for example, a mouse antibody, can then be systematically engineered into a human, humanized, or chimeric antibody of the invention.

For example, it has been observed that antibodies (*e.g.*, IgG antibodies) can be grouped into those found to exhibit tight, intermediate, or weak binding to an Fc receptor (*e.g.*, an Fc receptor on human monocytes (Fc γ RI)). By comparison of the amino-acid sequences in these different affinity groups, a monocyte-binding site in the hinge-link region (Leu234-Ser239) has been identified. Moreover, the human Fc γ RI receptor binds human IgG1 and mouse IgG2a as a monomer, but the binding of mouse IgG2b is 100-fold weaker. A comparison of the sequence of these proteins in the hinge-link region shows that the sequence 234 to 238, *i.e.*, Leu-Leu-Gly-Gly-Pro (SEQ ID NO:32) in the strong binders becomes Leu-Glu-Gly-Gly-Pro (SEQ ID NO:33) in mouse gamma 2b, *i.e.*, weak binders. Accordingly, a corresponding change in a human antibody hinge sequence can be made if reduced Fc γ I receptor binding is desired. It is understood that other alterations can be made to achieve the same or similar results. For example, the affinity of Fc γ RI binding can be altered by replacing the specified residue with a residue having an inappropriate functional group on its sidechain, or by introducing a charged functional group (*e.g.*, Glu or Asp) or for example an aromatic non-polar residue (*e.g.*, Phe, Tyr, or Trp).

These changes can be equally applied to the murine, human, and rat systems given the sequence homology between the different immunoglobulins. It has been shown that for human IgG3, which binds to the human Fc γ RI receptor, changing Leu 235 to Glu destroys the interaction of the mutant for the receptor. The binding site

for this receptor can thus be switched on or switched off by making the appropriate mutation.

Mutations on adjacent or close sites in the hinge link region (*e.g.*, replacing residues 234, 236 or 237 by Ala) indicate that alterations in residues 234, 235, 5 236, and 237 at least affect affinity for the Fc γ RI receptor. Accordingly, the antibodies of the invention can also have an altered Fc region with altered binding affinity for Fc γ RI as compared with the unmodified antibody. Such an antibody conveniently has a modification at amino acid residue 234, 235, 236, or 237.

Affinity for other Fc receptors can be altered by a similar approach, for 10 controlling the immune response in different ways.

As a further example, the lytic properties of IgG antibodies following binding of the Cl component of complement can be altered.

The first component of the complement system, Cl, comprises three proteins known as Clq, Clr and Cls which bind tightly together. It has been shown that 15 Clq is responsible for binding of the three protein complex to an antibody.

Accordingly, the Clq binding activity of an antibody can be altered by providing an antibody with an altered CH 2 domain in which at least one of the amino acid residues 318, 320, and 322 of the heavy chain has been changed to a residue having a different side chain. The numbering of the residues in the heavy chain is that of the 20 EU index (see Kabat *et al.*, *supra*). Other suitable alterations for altering, *e.g.*, reducing or abolishing specific Clq-binding to an antibody include changing any one of residues 318 (Glu), 320 (Lys) and 322 (Lys), to Ala.

Moreover, by making mutations at these residues, it has been shown that 25 Clq binding is retained as long as residue 318 has a hydrogen-bonding side chain and residues 320 and 322 both have a positively charged side chain.

Clq binding activity can be abolished by replacing any one of the three specified residues with a residue having an inappropriate functionality on its side chain. It is not necessary to replace the ionic residues only with Ala to abolish Clq binding. It is also possible to use other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or 30 Val, or such aromatic non-polar residues as Phe, Tyr, Trp and Pro in place of any one of the three residues in order to abolish Clq binding. In addition, it is also possible to use such polar non-ionic residues as Ser, Thr, Cys, and Met in place of residues 320 and 322, but not 318, in order to abolish Clq binding activity.

It is also noted that the side chains on ionic or non-ionic polar residues will be able to form hydrogen bonds in a similar manner to the bonds formed by the Glu residue. Therefore, replacement of the 318 (Glu) residue by a polar residue may modify but not abolish Clq binding activity.

5 It is also known that replacing residue 297 (Asn) with Ala results in removal of lytic activity while only slightly reducing (about three fold weaker) affinity for Clq. This alteration destroys the glycosylation site and the presence of carbohydrate that is required for complement activation. Any other substitution at this site will also destroy the glycosylation site.

10 The invention also provides an antibody having an altered effector function wherein the antibody has a modified hinge region. The modified hinge region may comprise a complete hinge region derived from an antibody of different antibody class or subclass from that of the CH1 domain. For example, the constant domain (CH1) of a class IgG antibody can be attached to a hinge region of a class IgG4 antibody.

15 Alternatively, the new hinge region may comprise part of a natural hinge or a repeating unit in which each unit in the repeat is derived from a natural hinge region. In one example, the natural hinge region is altered by converting one or more cysteine residues into a neutral residue, such as alanine, or by converting suitably placed residues into cysteine residues. Such alterations are carried out using art recognized protein chemistry
20 and, preferably, genetic engineering techniques, as described herein.

In one embodiment of the invention, the number of cysteine residues in the hinge region of the antibody is reduced, for example, to one cysteine residue. This modification has the advantage of facilitating the assembly of the antibody, for example, bispecific antibody molecules and antibody molecules wherein the Fc portion has been
25 replaced by an effector or reporter molecule, since it is only necessary to form a single disulfide bond. This modification also provides a specific target for attaching the hinge region either to another hinge region or to an effector or reporter molecule, either directly or indirectly, for example, by chemical means.

Conversely, the number of cysteine residues in the hinge region of the
30 antibody is increased, for example, at least one more than the number of normally occurring cysteine residues. Increasing the number of cysteine residues can be used to stabilize the interactions between adjacent hinges. Another advantage of this

modification is that it facilitates the use of cysteine thiol groups for attaching effector or reporter molecules to the altered antibody, for example, a radiolabel.

Accordingly, the invention provides for an exchange of hinge regions between antibody classes, in particular, IgG classes, and/or an increase or decrease in the 5 number of cysteine residues in the hinge region in order to achieve an altered effector function (see for example U.S. Patent No. 5,677,425 which is expressly incorporated herein). A determination of altered antibody effector function is made using the assays described herein or other art recognized techniques.

Importantly, the resultant antibody can be subjected to one or more 10 assays to evaluate any change in biological activity compared to the starting antibody. For example, the ability of the antibody with an altered Fc region to bind complement or Fc receptors can be assessed using the assays disclosed herein as well as any art recognized assay.

Production of the antibodies of the invention is carried out by any 15 suitable technique including techniques described herein as well as techniques known to those skilled in the art. For example an appropriate protein sequence, *e.g.* forming part of or all of a relevant constant domain, *e.g.*, Fc region, *i.e.*, CH₂, and/or CH₃ domain(s), of an antibody, and include appropriately altered residue(s) can be synthesized and then chemically joined into the appropriate place in an antibody molecule.

20 Preferably, genetic engineering techniques are used for producing an altered antibody. Preferred techniques include, for example, preparing suitable primers for use in polymerase chain reaction (PCR) such that a DNA sequence which encodes at least part of an IgG heavy chain, *e.g.*, an Fc or constant region (*e.g.*, CH₂, and/or CH₃) is altered, at one or more residues. The segment can then be operably linked to the 25 remaining portion of the antibody, *e.g.*, the variable region of the antibody and required regulatory elements for expression in a cell.

The present invention also includes vectors used to transform the cell line, vectors used in producing the transforming vectors, cell lines transformed with the transforming vectors, cell lines transformed with preparative vectors, and methods for 30 their production.

Preferably, the cell line which is transformed to produce the antibody with an altered Fc region (*i.e.*, of altered effector function) is an immortalized mammalian cell line (*e.g.*, CHO cell).

Although the cell line used to produce the antibody with an altered Fc region is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used.

5 *11. Affinity Maturation*

Antibodies (*e.g.*, humanized antibodies) of the invention can be modified for improved function using any of a number of affinity maturation techniques. Typically, a candidate molecule with a binding affinity to a given target molecule is identified and then further improved or “matured” using mutagenesis techniques 10 resulting in one or more related candidates having a more desired binding interaction with the target molecule. Typically, it is the affinity of the antibody (or avidity, *i.e.*, the combined affinities of the antibody for a target antigen) that is modified, however, other properties of the molecule, such as stability, effector function, clearance, secretion, or transport function, may also be modified, either separately or in parallel with affinity, 15 using affinity maturation techniques.

In exemplary embodiments, the affinity of an antibody (*e.g.*, a humanized antibody of the instant invention) is increased. For example, antibodies having binding affinities of at least $10^7 M^{-1}$, $10^8 M^{-1}$ or $10^9 M^{-1}$ can be matured such that their affinities are at least $10^9 M^{-1}$, $10^{10} M^{-1}$ or $10^{12} M^{-1}$.

20 One approach for affinity maturing a binding molecule is to synthesize a nucleic acid encoding the binding molecule, or portion thereof, that encodes the desired change or changes. Oligonucleotide synthesis is well known in the art and readily automated to produce one or more nucleic acids having any desired codon change(s). Restriction sites, silent mutations, and favorable codon usage may also be introduced in 25 this way. Alternatively, one or more codons can be altered to represent a subset of particular amino acids, *e.g.*, a subset that excludes cysteines which can form disulfide linkages, and is limited to a defined region, for example, a CDR region or portion thereof. Alternatively, the region may be represented by a partially or entirely random set of amino acids (for additional details, see, *e.g.*, U.S. Patent Nos. 5,830,650; 30 5,798,208; 5,824,514; 5,817,483; 5,814,476; 5,723,323; 4,528,266; 4,359,53; 5,840,479; and 5,869,644).

It is understood that the above approaches can be carried out in part or in full using polymerase chain reaction (PCR) which is well known in the art and has the advantage of incorporating oligonucleotides, *e.g.*, primers or single stranded nucleic acids having, *e.g.*, a desired alteration(s), into a double stranded nucleic acid and in amplified amounts suitable for other manipulations, such as genetic engineering into an appropriate expression or cloning vector. Such PCR can also be carried out under conditions that allow for misincorporation of nucleotides to thereby introduce additional variability into the nucleic acids being amplified. Experimental details for carrying out PCR and related kits, reagents, and primer design can be found, *e.g.*, in U.S. Pat. Nos. 4,683,202; 4,683,195; 6,040,166; and 6,096,551. Methods for introducing CDR regions into antibody framework regions using primer-based PCR is described in, *e.g.*, U.S. Patent No. 5,858,725. Methods for primer-based PCR amplification of antibody libraries (and libraries made according to method) employing a minimal set of primers capable of finding sequence homology with a larger set of antibody molecules, such that a larger and diverse set of antibody molecules can be efficiently amplified, is described, *e.g.*, in U.S. Pat. Nos. 5,780,225; 6,303,313; and 6,479,243. Non PCR-based methods for performing site directed mutagenesis can also be used and include ‘Kunkel’ mutagenesis that employs single-stranded uracil containing templates and primers that hybridize and introduce a mutation when passed through a particular strain of *E. coli* (see, *e.g.*, U.S. pat. No. 4,873,192).

Additional methods for varying an antibody sequence, or portion thereof, include nucleic acid synthesis or PCR of nucleic acids under nonoptimal (*i.e.*, error-prone) conditions, denaturation and renaturation (annealing) of such nucleic acids, exonuclease and/or endonuclease digestion followed by reassembly by ligation or PCR (nucleic acid shuffling), or a combination of one or more of the foregoing techniques as described, for example, in U.S. Pat. Nos. 6,440,668; 6,238,884; 6,171,820; 5,965,408; 6,361,974; 6,358,709; 6,352,842; 4,888,286; 6,337,186; 6,165,793; 6,132,970; 6,117,679; 5,830,721; and 5,605,793.

In certain embodiment, antibody libraries (or *affinity* maturation libraries) comprising a family of candidate antibody molecules having diversity in certain portions of the candidate antibody molecule, *e.g.*, in one or more CDR regions (or a portion thereof), one or more framework regions, and/or one or more constant regions (*e.g.*, a constant region having effector function) can be expressed and screened for desired

properties using art recognized techniques (see, e.g., U.S. Patent Nos. 6,291,161; 6,291,160; 6,291,159; and 6,291,158). For example, expression libraries of antibody variable domains having a diversity of CDR3 sequences and methods for producing human antibody libraries having a diversity of CDR3 sequences by introducing, by 5 mutagenesis, a diversity of CDR3 sequences and recovering the library can be constructed (see, e.g., U.S. Patent No. 6,248,516).

Finally, for expressing the affinity matured antibodies, nucleic acids encoding the candidate antibody molecules can be introduced into cells in an appropriate expression format, e.g., as full length antibody heavy and light chains (e.g., IgG), 10 antibody Fab fragments (e.g., Fab, F(ab')₂), or as single chain antibodies (scFv) using standard vector and cell transfection/transformation technologies (see, e.g., U.S. Patent Nos. 6,331,415; 6,103,889; 5,260,203; 5,258,498; and 4,946,778).

B. Nucleic Acid Encoding Immunologic and Therapeutic Agents

15 Immune responses against amyloid deposits can also be induced by administration of nucleic acids encoding antibodies and their component chains used for passive immunization. Such nucleic acids can be DNA or RNA. A nucleic acid segment encoding an immunogen is typically linked to regulatory elements, such as a promoter and enhancer, that allow expression of the DNA segment in the intended target 20 cells of a patient. For expression in blood cells, as is desirable for induction of an immune response, exemplary promoter and enhancer elements include those from light or heavy chain immunoglobulin genes and/or the CMV major intermediate early promoter and enhancer (Stinski, U.S. Patent Nos. 5,168,062 and 5,385,839). The linked regulatory elements and coding sequences are often cloned into a vector. For 25 administration of double-chain antibodies, the two chains can be cloned in the same or separate vectors.

A number of viral vector systems are available including retroviral systems (see, e.g., Lawrie and Tumin, *Cur. Opin. Genet. Develop.* 3:102-109 (1993)); adenoviral vectors (see, e.g., Bett *et al.*, *J. Virol.* 67:5911 (1993)); adeno-associated virus vectors (see, e.g., Zhou *et al.*, *J. Exp. Med.* 179:1867 (1994)), viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses (see, e.g., Dubensky *et al.*, *J. Virol.* 70:508 (1996)), Venezuelan equine encephalitis virus (see 30

Johnston *et al.*, US 5,643,576) and rhabdoviruses, such as vesicular stomatitis virus (see Rose, 6,168,943) and papillomaviruses (Ohe *et al.*, *Human Gene Therapy* 6:325 (1995); Woo *et al.*, WO 94/12629 and Xiao & Brandsma, *Nucleic Acids. Res.* 24, 2630-2622 (1996)).

5 DNA encoding an immunogen, or a vector containing the same, can be packaged into liposomes. Suitable lipids and related analogs are described by Eppstein *et al.*, US 5,208,036, Felgner *et al.*, US 5,264,618, Rose, US 5,279,833, and Epand *et al.*, US 5,283,185. Vectors and DNA encoding an immunogen can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate
10 polymers and polylactides and poly (lactide-co-glycolides), see, e.g., McGee *et al.*, *J. Micro Encap.* (1996).

Gene therapy vectors or naked polypeptides (e.g., DNA) can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, nasal, gastric, intradermal, intramuscular, subdermal,
15 or intracranial infusion) or topical application (see e.g., Anderson *et al.*, US 5,399,346). The term "naked polynucleotide" refers to a polynucleotide not delivered in association with a transfection facilitating agent. Naked polynucleotides are sometimes cloned in a plasmid vector. Such vectors can further include facilitating agents such as bupivacaine (Weiner *et al.*, US 5,593,972). DNA can also be administered using a gene gun. See
20 Xiao & Brandsma, *supra*. The DNA encoding an immunogen is precipitated onto the surface of microscopic metal beads. The microprojectiles are accelerated with a shock wave or expanding helium gas, and penetrate tissues to a depth of several cell layers. For example, The Accel™ Gene Delivery Device manufactured by Agricetus, Inc.
25 Middleton WI is suitable. Alternatively, naked DNA can pass through skin into the blood stream simply by spotting the DNA onto skin with chemical or mechanical irritation (see Howell *et al.*, WO 95/05853).

In a further variation, vectors encoding immunogens can be delivered to cells *ex vivo*, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed
30 by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

II. Prophylactic and Therapeutic Methods

The present invention is directed *inter alia* to treatment of Alzheimer's and other amyloidogenic diseases by administration of therapeutic immunological reagents (e.g., humanized immunoglobulins) to specific epitopes within A β to a patient under conditions that generate a beneficial therapeutic response in a patient (e.g., induction of phagocytosis of A β , reduction of plaque burden, inhibition of plaque formation, reduction of neuritic dystrophy, improving cognitive function, and/or reversing, treating or preventing cognitive decline) in the patient, for example, for the prevention or treatment of an amyloidogenic disease. The invention is also directed to use of the disclosed immunological reagents (e.g., humanized immunoglobulins) in the manufacture of a medicament for the treatment or prevention of an amyloidogenic disease.

In one aspect, the invention provides methods of preventing or treating a disease associated with amyloid deposits of A β in the brain of a patient. Such diseases include Alzheimer's disease, Down's syndrome and cognitive impairment. The latter can occur with or without other characteristics of an amyloidogenic disease. Some methods of the invention comprise administering an effective dosage of an antibody that specifically binds to a component of an amyloid deposit to the patient. Such methods are particularly useful for preventing or treating Alzheimer's disease in human patients. Exemplary methods comprise administering an effective dosage of an antibody that binds to A β . Preferred methods comprise administering an effective dosage of an antibody that specifically binds to an epitope within residues 1-10 of A β , for example, antibodies that specifically bind to an epitope within residues 1-3 of A β , antibodies that specifically bind to an epitope within residues 1-4 of A β , antibodies that specifically bind to an epitope within residues 1-5 of A β , antibodies that specifically bind to an epitope within residues 1-6 of A β , antibodies that specifically bind to an epitope within residues 1-7 of A β , or antibodies that specifically bind to an epitope within residues 3-7 of A β . In yet another aspect, the invention features administering antibodies that bind to an epitope comprising a free N-terminal residue of A β . In yet another aspect, the invention features administering antibodies that bind to an epitope within residues of 1-10 of A β wherein residue 1 and/or residue 7 of A β is aspartic acid. In yet another aspect, the invention features administering antibodies that specifically bind to A β .

peptide without binding to full-length amyloid precursor protein (APP). In yet another aspect, the isotype of the antibody is human IgG1.

In yet another aspect, the invention features administering antibodies that bind to an amyloid deposit in the patient and induce a clearing response against the amyloid deposit. For example, such a clearing response can be effected by Fc receptor mediated phagocytosis.

Therapeutic agents of the invention are typically substantially pure from undesired contaminant. This means that an agent is typically at least about 50% w/w (weight/weight) pure, as well as being substantially free from interfering proteins and contaminants. Sometimes the agents are at least about 80% w/w and, more preferably at least 90 or about 95% w/w pure. However, using conventional protein purification techniques, homogeneous peptides of at least 99% w/w pure can be obtained.

The methods can be used on both asymptomatic patients and those currently showing symptoms of disease. The antibodies used in such methods can be human, humanized, chimeric or nonhuman antibodies, or fragments thereof (*e.g.*, antigen binding fragments) and can be monoclonal or polyclonal, as described herein. In yet another aspect, the invention features administering antibodies prepared from a human immunized with A β peptide, which human can be the patient to be treated with antibody.

In another aspect, the invention features administering an antibody with a pharmaceutical carrier as a pharmaceutical composition. Alternatively, the antibody can be administered to a patient by administering a polynucleotide encoding at least one antibody chain. The polynucleotide is expressed to produce the antibody chain in the patient. Optionally, the polynucleotide encodes heavy and light chains of the antibody. The polynucleotide is expressed to produce the heavy and light chains in the patient. In exemplary embodiments, the patient is monitored for level of administered antibody in the blood of the patient.

The invention thus fulfills a longstanding need for therapeutic regimes for preventing or ameliorating the neuropathology and, in some patients, the cognitive impairment associated with Alzheimer's disease.

A. Patients Amenable to Treatment

Patients amenable to treatment include individuals at risk of disease but not showing symptoms, as well as patients presently showing symptoms. In the case of Alzheimer's disease, virtually anyone is at risk of suffering from Alzheimer's disease if he or she lives long enough. Therefore, the present methods can be administered prophylactically to the general population without the need for any assessment of the risk of the subject patient. The present methods are especially useful for individuals who have a known genetic risk of Alzheimer's disease. Such individuals include those having relatives who have experienced this disease, and those whose risk is determined by analysis of genetic or biochemical markers. Genetic markers of risk toward Alzheimer's disease include mutations in the APP gene, particularly mutations at position 717 and positions 670 and 671 referred to as the Hardy and Swedish mutations respectively (see Hardy, *supra*). Other markers of risk are mutations in the presenilin genes, PS1 and PS2, and ApoE4, family history of AD, hypercholesterolemia or atherosclerosis. Individuals presently suffering from Alzheimer's disease can be recognized from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have AD. These include measurement of CSF tau and A β 42 levels. Elevated tau and decreased A β 42 levels signify the presence of AD. Individuals suffering from Alzheimer's disease can also be diagnosed by ADRDA criteria as discussed in the Examples section.

In asymptomatic patients, treatment can begin at any age (e.g., 10, 20, 30). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70. Treatment typically involves multiple dosages over a period of time. Treatment can be monitored by assaying antibody levels over time. If the response falls, a booster dosage is indicated. In the case of potential Down's syndrome patients, treatment can begin antenatally by administering therapeutic agent to the mother or shortly after birth.

B. Treatment Regimes and Dosages

In prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, Alzheimer's disease in an amount sufficient to eliminate or reduce the risk, lessen the

severity, or delay the onset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. In therapeutic applications, compositions or medicaments are administered to a patient suspected of, or already 5 suffering from such a disease in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease.

In some methods, administration of agent reduces or eliminates myocognitive impairment in patients that have not yet developed characteristic 10 Alzheimer's pathology. An amount adequate to accomplish therapeutic or prophylactic treatment is defined as a therapeutically- or prophylactically-effective dose. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient immune response has been achieved. The term "immune response" or "immunological response" includes the development of a humoral (antibody mediated) 15 and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against an antigen in a recipient subject. Such a response can be an active response, *i.e.*, induced by administration of immunogen, or a passive response, *i.e.*, induced by administration of immunoglobulin or antibody or primed T-cells. Typically, the immune response is monitored and repeated dosages are given if the 20 immune response starts to wane.

Effective doses of the compositions of the present invention, for the treatment of the above described conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether 25 treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages need to be titrated to optimize safety and efficacy.

For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg (*e.g.*, 0.02 mg/kg, 0.25 mg/kg, 30 0.5 mg/kg, 0.75 mg/kg, 1mg/kg, 2 mg/kg, etc.), of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, preferably at least 1 mg/kg. In another example, dosages can be 0.5 mg/kg body weight or 15 mg/kg body weight or within the range of 0.5-15 mg/kg, preferably at

least 1 mg/kg. Doses intermediate in the above ranges are also intended to be within the scope of the invention. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment involves administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes involve administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to A β in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μ g/ml and in some methods 25-300 μ g/ml. Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, humanized antibodies show the longest half-life, followed by chimeric antibodies and nonhuman antibodies.

The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactic effective dose." In this use, the precise amounts again depend upon the patient's state of health and general immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per dose. A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives.

In therapeutic applications, a relatively high dosage (*e.g.*, from about 1 to 200 mg of antibody per dose, with dosages of from 5 to 25 mg being more commonly used) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete

amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

Doses for nucleic acids encoding antibodies range from about 10 ng to 1 g, 100 ng to 100 mg, 1 µg to 10 mg, or 30-300 µg DNA per patient. Doses for 5 infectious viral vectors vary from 10-100, or more, virions per dose.

Therapeutic agents can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intraperitoneal, intranasal or intramuscular means for prophylactic and/or therapeutic treatment. The most typical route of administration of an immunogenic agent is subcutaneous although other routes 10 can be equally effective. The next most common route is intramuscular injection. This type of injection is most typically performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example intracranial injection. Intramuscular injection or intravenous infusion are preferred for administration of antibody. In some methods, particular 15 therapeutic antibodies are injected directly into the cranium. In some methods, antibodies are administered as a sustained release composition or device, such as a Medipad™ device.

Agents of the invention can optionally be administered in combination with other agents that are at least partly effective in treatment of amyloidogenic disease. 20 In certain embodiments, a humanized antibody of the invention (*e.g.*, humanized 12A11) is administered in combination with a second immunogenic or immunologic agent. For example, a humanized 12A11 antibody of the invention can be administered in combination with another humanized antibody to A β . In other embodiments, a humanized 12A11 antibody is administered to a patient who has received or is receiving 25 an A β vaccine. In the case of Alzheimer's and Down's syndrome, in which amyloid deposits occur in the brain, agents of the invention can also be administered in conjunction with other agents that increase passage of the agents of the invention across the blood-brain barrier. Agents of the invention can also be administered in combination with other agents that enhance access of the therapeutic agent to a target cell or tissue, 30 for example, liposomes and the like. Coadministering such agents can decrease the dosage of a therapeutic agent (*e.g.*, therapeutic antibody or antibody chain) needed to achieve a desired effect.

C. Pharmaceutical Compositions

Agents of the invention are often administered as pharmaceutical compositions comprising an active therapeutic agent, *i.e.*, and a variety of other pharmaceutically acceptable components. See *Remington's Pharmaceutical Science* 5 (15th ed., Mack Publishing Company, Easton, Pennsylvania (1980)). The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The 10 diluent is selected so as not to affect the biological activity of the combination.

Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

15 Pharmaceutical compositions can also include large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polylactic acids, polyglycolic acids and copolymers (such as latex functionalized Sepharose (TM), agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Additionally, these carriers can 20 function as immunostimulating agents (*i.e.*, adjuvants).

For parenteral administration, agents of the invention can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or 25 emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Antibodies can be 30 administered in the form of a depot injection or implant preparation, which can be formulated in such a manner as to permit a sustained release of the active ingredient. An exemplary composition comprises monoclonal antibody at 5 mg/mL, formulated in

aqueous buffer consisting of 50 mM L-histidine, 150 mM NaCl, adjusted to pH 6.0 with HCl.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above (see Langer, *Science* 249: 1527 (1990) and Hanes, *Advanced Drug Delivery Reviews* 28:97 (1997)). The agents of this invention can be administered in the form of a depot injection or implant preparation, which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

Additional formulations suitable for other modes of administration include oral, intranasal, and pulmonary formulations, suppositories, and transdermal applications. For suppositories, binders and carriers include, for example, polyalkylene glycols or triglycerides; such suppositories can be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include excipients, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

Topical application can result in transdermal or intradermal delivery. Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof or other similar bacterial toxins (See 25 Glenn *et al.*, *Nature* 391, 851 (1998)). Co-administration can be achieved by using the components as a mixture or as linked molecules obtained by chemical crosslinking or expression as a fusion protein.

Alternatively, transdermal delivery can be achieved using a skin patch or using transferosomes (Paul *et al.*, *Eur. J. Immunol.* 25:3521 (1995); Cevc *et al.*, 30 *Biochem. Biophys. Acta* 1368:201-15 (1998)).

D. Monitoring the Course of Treatment

The invention provides methods of monitoring treatment in a patient suffering from or susceptible to Alzheimer's, *i.e.*, for monitoring a course of treatment being administered to a patient. The methods can be used to monitor both therapeutic treatment on symptomatic patients and prophylactic treatment on asymptomatic patients. 5 In particular, the methods are useful for monitoring passive immunization (*e.g.*, measuring level of administered antibody).

Some methods involve determining a baseline value, for example, of an antibody level or profile in a patient, before administering a dosage of agent, and 10 comparing this with a value for the profile or level after treatment. A significant increase (*i.e.*, greater than the typical margin of experimental error in repeat measurements of the same sample, expressed as one standard deviation from the mean of such measurements) in value of the level or profile signals a positive treatment outcome (*i.e.*, that administration of the agent has achieved a desired response). If the 15 value for immune response does not change significantly, or decreases, a negative treatment outcome is indicated.

In other methods, a control value (*i.e.*, a mean and standard deviation) of level or profile is determined for a control population. Typically the individuals in the control population have not received prior treatment. Measured values of the level or 20 profile in a patient after administering a therapeutic agent are then compared with the control value. A significant increase relative to the control value (*e.g.*, greater than one standard deviation from the mean) signals a positive or sufficient treatment outcome. A lack of significant increase or a decrease signals a negative or insufficient treatment outcome. Administration of agent is generally continued while the level is increasing 25 relative to the control value. As before, attainment of a plateau relative to control values is an indicator that the administration of treatment can be discontinued or reduced in dosage and/or frequency.

In other methods, a control value of the level or profile (*e.g.*, a mean and standard deviation) is determined from a control population of individuals who have 30 undergone treatment with a therapeutic agent and whose levels or profiles have plateaued in response to treatment. Measured values of levels or profiles in a patient are compared with the control value. If the measured level in a patient is not significantly different (*e.g.*, more than one standard deviation) from the control value, treatment can

be discontinued. If the level in a patient is significantly below the control value, continued administration of agent is warranted. If the level in the patient persists below the control value, then a change in treatment may be indicated.

In other methods, a patient who is not presently receiving treatment but 5 has undergone a previous course of treatment is monitored for antibody levels or profiles to determine whether a resumption of treatment is required. The measured level or profile in the patient can be compared with a value previously achieved in the patient after a previous course of treatment. A significant decrease relative to the previous measurement (*i.e.*, greater than a typical margin of error in repeat measurements of the 10 same sample) is an indication that treatment can be resumed. Alternatively, the value measured in a patient can be compared with a control value (mean plus standard deviation) determined in a population of patients after undergoing a course of treatment. Alternatively, the measured value in a patient can be compared with a control value in populations of prophylactically treated patients who remain free of symptoms of disease, 15 or populations of therapeutically treated patients who show amelioration of disease characteristics. In all of these cases, a significant decrease relative to the control level (*i.e.*, more than a standard deviation) is an indicator that treatment should be resumed in a patient.

The tissue sample for analysis is typically blood, plasma, serum, mucous 20 fluid or cerebrospinal fluid from the patient. The sample is analyzed, for example, for levels or profiles of antibodies to A β peptide, *e.g.*, levels or profiles of humanized antibodies. ELISA methods of detecting antibodies specific to A β are described in the Examples section. In some methods, the level or profile of an administered antibody is determined using a clearing assay, for example, in an *in vitro* phagocytosis assay, as 25 described herein. In such methods, a tissue sample from a patient being tested is contacted with amyloid deposits (*e.g.*, from a PDAPP mouse) and phagocytic cells bearing Fc receptors. Subsequent clearing of the amyloid deposit is then monitored. The existence and extent of clearing response provides an indication of the existence and level of antibodies effective to clear A β in the tissue sample of the patient under test.

30 The antibody profile following passive immunization typically shows an immediate peak in antibody concentration followed by an exponential decay. Without a further dosage, the decay approaches pretreatment levels within a period of days to months depending on the half-life of the antibody administered.

In some methods, a baseline measurement of antibody to A β in the patient is made before administration, a second measurement is made soon thereafter to determine the peak antibody level, and one or more further measurements are made at intervals to monitor decay of antibody levels. When the level of antibody has declined 5 to baseline or a predetermined percentage of the peak less baseline (e.g., 50%, 25% or 10%), administration of a further dosage of antibody is administered. In some methods, peak or subsequent measured levels less background are compared with reference levels previously determined to constitute a beneficial prophylactic or therapeutic treatment regime in other patients. If the measured antibody level is significantly less than a 10 reference level (e.g., less than the mean minus one standard deviation of the reference value in population of patients benefiting from treatment) administration of an additional dosage of antibody is indicated.

Additional methods include monitoring, over the course of treatment, any art-recognized physiologic symptom (e.g., physical or mental symptom) routinely relied 15 on by researchers or physicians to diagnose or monitor amyloidogenic diseases (e.g., Alzheimer's disease). For example, one can monitor cognitive impairment. The latter is a symptom of Alzheimer's disease and Down's syndrome but can also occur without other characteristics of either of these diseases. For example, cognitive impairment can be monitored by determining a patient's score on the Mini-Mental State Exam in 20 accordance with convention throughout the course of treatment.

E. Kits

The invention further provides kits for performing the monitoring methods described above. Typically, such kits contain an agent that specifically binds to 25 antibodies to A β . The kit can also include a label. For detection of antibodies to A β , the label is typically in the form of labeled anti-idiotypic antibodies. For detection of antibodies, the agent can be supplied prebound to a solid phase, such as to the wells of a microtiter dish. Kits also typically contain labeling providing directions for use of the kit. The labeling may also include a chart or other correspondence regime correlating 30 levels of measured label with levels of antibodies to A β . The term labeling refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. For example, the term labeling

encompasses advertising leaflets and brochures, packaging materials, instructions, audio or videocassettes, computer discs, as well as writing imprinted directly on kits.

The invention also provides diagnostic kits, for example, research, detection and/or diagnostic kits (*e.g.*, for performing *in vivo* imaging). Such kits 5 typically contain an antibody for binding to an epitope of A β , preferably within residues 1-10. Preferably, the antibody is labeled or a secondary labeling reagent is included in the kit. Preferably, the kit is labeled with instructions for performing the intended application, for example, for performing an *in vivo* imaging assay. Exemplary antibodies are those described herein.

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F. *In vivo* Imaging

The invention provides methods of *in vivo* imaging amyloid deposits in a patient. Such methods are useful to diagnose or confirm diagnosis of Alzheimer's disease, or susceptibility thereto. For example, the methods can be used on a patient 15 presenting with symptoms of dementia. If the patient has abnormal amyloid deposits, then the patient is likely suffering from Alzheimer's disease. The methods can also be used on asymptomatic patients. Presence of abnormal deposits of amyloid indicates susceptibility to future symptomatic disease. The methods are also useful for monitoring disease progression and/or response to treatment in patients who have been previously 20 diagnosed with Alzheimer's disease.

The methods work by administering a reagent, such as antibody that binds to A β , to the patient and then detecting the agent after it has bound. Preferred antibodies bind to A β deposits in a patient without binding to full length APP polypeptide. Antibodies binding to an epitope of A β within amino acids 1-10 are particularly 25 preferred. In some methods, the antibody binds to an epitope within amino acids 7-10 of A β . Such antibodies typically bind without inducing a substantial clearing response. In other methods, the antibody binds to an epitope within amino acids 1-7 of A β . Such antibodies typically bind and induce a clearing response to A β . However, the clearing response can be avoided by using antibody fragments lacking a full-length constant 30 region, such as Fabs. In some methods, the same antibody can serve as both a treatment and diagnostic reagent. In general, antibodies binding to epitopes C-terminal to residue 10 of A β do not show as strong a signal as antibodies binding to epitopes within residues

1-10, presumably because the C-terminal epitopes are inaccessible in amyloid deposits. Accordingly, such antibodies are less preferred.

Diagnostic reagents can be administered by intravenous injection into the body of the patient, or directly into the brain by intracranial injection or by drilling a
5 hole through the skull. The dosage of reagent should be within the same ranges as for treatment methods. Typically, the reagent is labeled, although in some methods, the primary reagent with affinity for A β is unlabelled and a secondary labeling agent is used to bind to the primary reagent. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical detection. Use of paramagnetic
10 labels is suitable for tomographic detection without surgical intervention. Radioactive labels can also be detected using PET or SPECT.

Diagnosis is performed by comparing the number, size, and/or intensity of labeled loci, to corresponding baseline values. The base line values can represent the mean levels in a population of undiseased individuals. Baseline values can also
15 represent previous levels determined in the same patient. For example, baseline values can be determined in a patient before beginning treatment, and measured values thereafter compared with the baseline values. A decrease in values relative to baseline signals a positive response to treatment.

20 The present invention will be more fully described by the following non-limiting examples.

EXAMPLES

The following Sequence identifiers are used throughout the Examples section to refer to immunoglobulin chain variable region nucleotide and amino acid sequences.

5

<u>Antibody</u>	<u>VL nucleotide sequence</u>	<u>VL amino acid sequence</u>	<u>VH nucleotide sequence</u>	<u>VH amino acid sequence</u>
12A11	SEQ ID NO:1 (coding)	SEQ ID NO:2	SEQ ID NO:3 (coding)	SEQ ID NO:4
12A11v1	SEQ ID NO:34	SEQ ID NO:7	SEQ ID NO:35	SEQ ID NO:10
12A11v2		SEQ ID NO:7		SEQ ID NO:13
12A11v2.1		SEQ ID NO:7		SEQ ID NO:14
12A11v3		SEQ ID NO:7		SEQ ID NO:15
12A11	SEQ ID NO:5		SEQ ID NO:6	

As used herein, an antibody or immunoglobulin sequence comprising a
10 VL and/or VH sequence as set forth in any one of SEQ ID NOs: 1-4 can comprise either the full sequence or can comprise the mature sequence (*i.e.*, mature peptide without the signal or leader peptide).

Previous studies have shown that it is possible to predict *in vivo* efficacy of
15 various A β antibodies in reducing AD-associated neuropathology (*e.g.*, plaque burden) by the ability of antibodies to bind plaques *ex vivo* (*e.g.*, in PDAPP or AD brain sections) and/or trigger plaque clearance in an *ex vivo* phagocytosis assay (Bard *et al.* (2000) *Nat. Med.* 6:916-919). The correlation supports the notion that Fc-dependent phagocytosis by microglial cells and/or macrophages is important to the process of
20 plaque clearance *in vivo*. However, it has also been reported that antibody efficacy can also be obtained *in vivo* by mechanisms that are independent of Fc interactions (Bacskaï *et al.* (2002) *J. Neurosci.* 22:7873-7878). Studies have indicated that an antibody directed against the midportion of A β , which cannot recognize amyloid plaques, appears

to bind to soluble A β and reduce plaque deposition (DeMattos *et al.* (2001) *Proc. Natl. Acad. Sci. USA* 98:8850-8855).

5 In order to characterize potential *in vivo* efficacy of the murine monoclonal antibody 12A11 (isotype IgG1), various *ex vivo* assays were first performed.

Avidity of mAb 12A11 for A β 1-42. Binding of monoclonal antibody 12A11 to aggregated synthetic A β 1-42 was performed by ELISA, as described in Schenk, *et al.* (*Nature* 400:173 (1999)). For comparison purposes, mAbs 12B4, and 10D5 were also assayed. Soluble A β 1-42 refers to the synthetic A β 1-42 peptide sonicated in dimethyl sulfoxide (DMSO). Serial dilutions of the antibodies at 20 μ g/ml were incubated with 50,000 cpm [125 I]A β 1-42 (190 μ Ci/ μ mol; labeling with Iodogen reagent, Pierce) overnight at room temperature. Fifty microliters of a slurry containing 75 mg/ml protein A Sepharose (Amersham Pharmacia) and 200 μ g of rabbit anti-mouse IgG (H+L) (Jackson ImmunoResearch) were incubated with the diluted antibodies for 1 h at room temperature, washed twice, and counted on a Wallac gamma counter (Perkin-Elmer). All steps were performed in RIA buffer consisting of 10 mM Tris, 0.5 M NaCl, 1 mg/ml gelatin, and 0.5% Nonidet P-40, pH 8.0.

10 Results from the avidity study are shown below in Table 2.
 15

20

Table 2.

<u>Antibody</u>	<u>Epitope</u>	<u>Isotype</u>	<u>ED₅₀ on aggregated Aβ1-42, pM</u>	<u>% Capture of soluble Aβ1-42</u>
10D5 [†]	A β 3-7	IgG1	53	1
12B4 [†]	A β 3-7	IgG2a	667	8
12A11	A β 3-7	IgG1	233	30

[†]Antibodies 10D5 and 12B4 are described in detail in WO 02/46237 and International Patent Application Serial No. PCT/US03/07715, respectively.

25 * As a comparison, the antibody 266 at 10 μ g/ml would capture 70% of A β 1-42.

All of the antibodies tested exhibited a high avidity for aggregated A β 1-42.

Moreover, antibodies 12B4 and 12A11 appreciably captured soluble A β 1-42 at antibody concentrations of 20 μ g/ml. As shown in Table 2, the IgG1 antibody 12A11 captured A β 1-42 more efficiently than the IgG2a antibody 12B4 or the IgG1 antibody 10D5.

5 The ability of various antibodies (including 12A11) to capture soluble A β was further assayed as follows. Various concentrations of antibody (up to 10 μ g/ml) were incubated with 50,000 CPM of 125 I-A β 1-42 (or 125 I-A β 1-40). The concentration of antibody sufficient to bind 25% of the radioactive counts was determined in a capture radioimmunoassay. For antibodies not capable of binding 25% of the counts at 10 μ g/ml, the percentage of counts bound at 10 μ g/ml was determined. The 12A11 bound 20% of the radioactive counts (*i.e.*, 125 I-A β) at 10 μ g/ml. This was greater than the amount bound by two other A β 3-7 antibodies tested, namely 12B4 and 10D5 (binding 7% and 2% at 10 μ g/ml, respectively). Thus, of the N-terminal (epitope A β 3-7) antibodies tested, 12A11 exhibited the most appreciable ability to capture A β .

10 As a measure of their ability to trigger Fc-mediated plaque clearance, the antibodies were also compared in an *ex vivo* phagocytosis assay with primary mouse microglial cells and sections of brain tissue from PDAPP mice. Irrelevant IgG1 and IgG2a antibodies, having no reactivity toward A β or other components of the assay, were used as isotype-matched negative controls. Briefly, murine primary microglial 15 cells were cultured with unfixed cryostat sections of PDAPP mouse brain in the presence of antibodies. After 24 h of incubation, the total level of A β remaining in the cultures was measured by ELISA. To quantify the degree of plaque clearance/A β degradation, A β was extracted from the cultures of microglia and brain sections ($n = 3$) with 8 M urea 20 for ELISA analysis. Data were analyzed with ANOVA followed by a post hoc 25 Dunnett's test.

As shown in Figure 1, the 12B4 antibody reduced A β levels efficiently (73% for 12B4; P < 0.001) with 12A11 showing somewhat less, albeit statistically significant, efficiency (48% for 12A11, P < 0.05). The 10D5 antibody did not significantly reduce A β levels. The performance of 12A11 in the *ex vivo* phagocytosis assay may be 30 improved upon conversion to the IgG2a isotype which is a preferred isotype for microglial phagocytosis.

Example II. *In vivo* Efficacy of Mouse 12A11 Antibody

Mouse Antibody 12A11 Reduces Alzheimer's-Like Neuropathology In Vivo To determine the *in vivo* efficacy of 12A11, antibodies (including 12A11, 12B4, or 10D5) were administered to mice at 10 mg/kg by weekly intraperitoneal injection for 6 months as described in Bard *et al.* (2000) *Nat. Med.* 6:916. At the end of the study, total levels of cortical A β were determined by ELISA. As shown in Figure 2A, each of the antibodies significantly reduced total A β levels compared with the PBS control (P < 0.001), *i.e.* 12B4 showed a 69% reduction, 10D5 showed a 52% reduction, and 12A11 showed a 31% reduction.

The level of neuritic dystrophy was then examined in sections of brain tissue from the above-mentioned mice to determine the association between plaque clearance and neuronal protection. Data from brain image analyses examining the percentage of frontal cortex occupied by neurotic dystrophy is shown in Figure 2B. These data show that antibodies 10D5 and 12A11 were not effective at reducing neuritic dystrophy whereas 12B4 significantly reduced neuritic dystrophy (12B4, P < 0.05; ANOVA followed by post hoc Dunnett's test), as determined by the assay described herein. Again, this activity of 12A11 may be improved by converting 12 A11 to the IgG2a isotype (murine efficacy). Regarding humanized versions of 12A11, IgG1 isotypes are preferred for reducing neuritic dystrophy.

Experiments demonstrating the binding properties and *in vivo* efficacy of antibody 12A11 are also described in Bard, *et al.* *PNAS* 100:2023 (2003), incorporated by reference herein.

In summary, all antibodies had significant avidity for aggregated A β and triggered plaque clearance in an *ex vivo* assay. The IgG2a isotype (affinity for Fc receptors, in particular, Fc γ RI) appears to be an important attribute for both clearance of A β and protection against neuritic dystrophy. The antibody 12A11 (IgG1) captured soluble monomeric A β 1-42 more efficiently than 12B4 (IgG2a) or 10D5 (IgG1) but was not as effective at reducing neuritic dystrophy. Enhanced efficacy in reducing plaque burden and reducing neuritic dystrophy may be achieved by engineering antibodies to have an isotype which maximally supports phagocytosis. Particularly efficacious antibodies bind to epitopes within the N terminus of A β .

Example III. Cloning and Sequencing of the Mouse 12A11 Variable Regions

Cloning and Sequence Analysis of 12A11 VH. The VH and VL regions of 12A11 from hybridoma cells were cloned by RT-PCR and 5' RACE using mRNA from hybridoma cells and standard cloning methodology. The nucleotide sequence (coding, SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) derived from independent cDNA clones encoding the presumed 12A11 VH domain, are set forth in Table 3 and Table 4, respectively.

10 Table 3: Mouse 12A11 VH DNA sequence.

```
ATGGACAGGCTTACTACTTCATTCCCTGCTGCTGATTGTCCCTGCATATGTCTT
GTCCCAAGTTACTCTAAAAGAGTCTGGCCCTGGGATATTGAAGCCCTCACAG
ACCCTCAGTCTGACTTGTTCTTCTCTGGGTTTCACTGAGCACTCTGGTAT
GAGTGTAGGCTGGATTCGTCAGCCTCAGGAAAGGGTCTGGAGTGGCTGGC
15 ACACATTTGGTGGGATGATGATAAGTACTATAACCCATCCCTGAAGAGCCGG
CTCACAACTCCAAGGATACCTCCAGAAACCAGGTATTCCCTCAAGATCACCA
GTGTGGACACTGCGAGATACTGCCACTTACTACTGTGCTCGAACGAAACTACTAC
GGCTGACTACTTGCCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
(SEQ ID NO:3)
```

20

Table 4: Mouse 12A11 VH amino acid sequence

```
mdrlttsfllivpayvlsvQVTLKESGPGILKPSQLSLTCSFSGFSLStsgmsvgWIRQPSGKG
LEWLAhiwwdddkyynpslksRLTISKDTSRNQVFLKITSVDTADTATYYCARrtttadyfa
yWGQGTTLTSS(SEQ ID NO: 4)
```

25 * Leader peptide and CDRs in lower case.

Cloning and Sequence Analysis of 12A11 VL. The light chain variable VL region of 12A11 was cloned in an analogous manner as the VH region. The nucleotide sequence (coding, SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) derived from two independent cDNA clones encoding the presumed 12A11 VL domain, are set forth in Table 5 and Table 6, respectively.

Table 5: Mouse 12A11 VL DNA sequence

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCCTGCTTCCAG
 CAGTGATGTTGATGACCCAACTCCACTCTCCCTGCCTGTCAGTCTTGGAG
 ATCAAGCCTCCATCTCTGCAGATCTAGTCAGAGCATTGTACATAGTAATGG
 5 AACACCTACTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCT
 CCTGATCTACAAAGTTCCAACCGATTTCTGGGGTCCCAGACAGGTTAGT
 GGCAGTGGATCAGGGACAGATTCACACTCAAGATCAGCAGAGTGGAGGCT
 GAGGATCTGGGAATTATTACTGCTTCAAAGTCACATGTTCTCACCGTT
 CGGTGCTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO:1)

10

Table 6: Mouse 12A11 VL amino acid sequence

mklpvrlvlvlfwipasssDVLMTQTPLSLPVSLGDQASISCrssqsivhsngntyleWYLQKPGQ
 SPKLLIYkvsnrfSGVPDRFSGSGSGTDFTLKRISRVEAEDLGIYYCfqsshvpltFGAGTK
 15 LELK (SEQ ID NO: 2)

* Leader peptide and CDRs in lower case.

The 12A11 VL and VH sequences meet the criteria for functional V regions in so far as they contain a contiguous ORF from the initiator methionine to the 20 C-region, and share conserved residues characteristic of immunoglobulin V region genes. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

Example IV: Expression of Chimeric 12A11 Antibody

25 *Expression of Chimeric 12A11 Antibody:* The variable heavy and light chain regions were re-engineered to encode splice donor sequences downstream of the respective VDJ or VJ junctions, and cloned into the mammalian expression vector pCMV-hy1 for the heavy chain, and pCMV-hκ1 for the light chain. These vectors encode human γ 1 and Cκ constant regions as exonic fragments downstream of the 30 inserted variable region cassette. Following sequence verification, the heavy chain and light chain expression vectors were co-transfected into COS cells. Various heavy chain

clones were independently co-transfected with different chimeric light chain clones to confirm reproducibility of the result. Antibodies were immunoprecipitated from COS cell conditioned media using protein A Sepharose. Antibody chains were detected on immunoblots of SDS-PAGE gels. Detection was accomplished using goat-anti-human-IgG (H+L) antibody at a 1:5000 dilution at room temperature for 1 hour. Significant quantities of 12A11 H+L chain were detected in conditioned media.

5 Direct binding of chimeric 12A11 antibody to A β was tested by ELISA assay. Figure 4 demonstrates that chimeric 12A11 was found to bind to A β with high avidity, similar to that demonstrated by chimeric and humanized 3D6. (The cloning, 10 characterization and humanization of 3D6 is described in U.S. Patent Application Serial No. 10/010,942, the entire content of which is incorporated herein by this reference.) Binding avidity was also similar to that demonstrated by chimeric and humanized 12B4. (The cloning, characterization and humanization of 12B4 is described in U.S. Patent Application Serial No. 10/388,214, the entire content of which is incorporated herein by 15 this reference.)

Example V. 12A11 Humanization

A. 12A11 Humanized Antibody, Version 1

20 *Homology/Molecular Model Analysis.* In order to identify key structural framework residues in the murine 12A11 antibody, three-dimensional models were studied for solved murine antibodies having homology to the 12A11 heavy and light chains. An antibody designated 1KTR was chosen having close homology to the 12A11 light chain and two antibodies designated 1ETZ and 1JRH were chosen having close homology to the 12A11 heavy chain. These mouse antibodies show strong sequence 25 conservation with 12A11 (94% identity in 112 amino acids for Vk and 83% identity in 126 amino acids and 86% identity in 121 amino acids respectively for Vh). The heavy chain structure of 1ETZ was superimposed onto that of 1KTR. In addition, for Vk the CDR loops of the selected antibody fall into the same canonical Chothia structural classes as do the CDR loops of 12A11 VL. The crystal structures of these antibodies 30 were examined for residues (e.g., FR residues important for CDR conformation, etc.) predicted be important for function of the antibody, and by comparison, function of the similar 12A11 antibody

- Selection of Human Acceptor Antibody Sequences.* Suitable human acceptor antibody sequences were identified by computer comparisons of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies. The comparison was performed separately for the 12A11 heavy and light chains. In 5 particular, variable domains from human antibodies whose framework sequences exhibited a high degree of sequence identity with the murine VL and VH framework regions were identified by query of the NCBI Ig Database using NCBI BLAST (publicly accessible through the National Institutes of Health NCBI internet server) with the respective murine framework sequences.
- 10 Two candidate sequences were chosen as acceptor sequences based on the following criteria: (1) homology with the subject sequence; (2) sharing canonical CDR structures with the donor sequence; and/or (3) not containing any rare amino acid residues in the framework regions. The selected acceptor sequence for VL is BAC01733 in the NCBI Ig non-redundant database. The selected acceptor sequence for 15 VH is AAA69734 in the NCBI Ig non-redundant database. AAA69734 is a human subgroup III antibody (rather than subgroup II) but was selected as an initial acceptor antibody based at least in part on the reasoning in Saldanha *et al.* (1999) *Mol. Immunol.* 36:709. First versions of humanized 12A11 antibody utilize these selected acceptor antibody sequences. The antibody is described in Schroeder and Wang (1990) *Proc. 20 Natl. Acad. Sci. USA* 87:6146.

Substitution of Amino Acid Residues. As noted *supra*, the humanized antibodies of the invention comprise variable framework regions substantially from a human immunoglobulin (acceptor immunoglobulin) and complementarity determining 25 regions substantially from a mouse immunoglobulin (donor immunoglobulin) termed 12A11. Having identified the complementarity determining regions of 12A11 and appropriate human acceptor immunoglobulins, the next step was to determine which, if any, residues from these components to substitute to optimize the properties of the resulting humanized antibody.

Reshaped light chain V region:

The amino acid alignment of the reshaped light chain V region is shown in Figure 5A. The choice of the acceptor framework (BAC01733) is from the same human subgroup as that which corresponds to the murine V region, has no unusual framework residues, and the CDRs belong to the same Chothia canonical structure groups. No backmutations were made in Version 1 of humanized 12A11
5

Reshaped heavy chain V region:

The amino acid alignment of the reshaped heavy chain V region is shown in Figure 5B. The choice for the acceptor framework (AAA69734) is from human subgroup III (as described previously) and has no unusual framework residues.
10 Structural analysis of the murine VH chain (1ETZ and 1JRH), in conjunction with the amino acid alignment of AAA69734 to the murine sequence dictates 9 backmutations in version 1 (v1) of the reshaped heavy chain: A24F T28S F29L V37I V48L F67L R71K N73T L78V (Kabat numbering). The back mutations are highlighted by asterisks in the
15 amino-acid alignment shown in Figure 5B.

Of the 9 back mutations, 3 are dictated by the model because the residues are canonical residues (A24F, F29L, & R71K, solid fill), *i.e.* framework residues which may contribute to antigen binding by virtue of proximity to CDR residues. There is one back mutation in the next most important class of residues, the interface residues involved in
20 VH-VL packing interactions (underlined), *i.e.*, V37I. The N73T mutation is at a vernier residue (dotted fill) on the edge of the binding site, possibly interacting with S30 adjacent to CDR1. The remaining 4 residues targeted for back mutation (T28S, V48L, F67L, L78V, Kabat numbering) also fall into the vernier class (indirect contribution to CDR conformation, dotted fill in Figure 5B).

25 A summary of the changes incorporated into version 1 of humanized 12A11 is presented in Table 7.

Table 7. Summary of changes in humanized 12A11.v1

Changes	VL (112 residues)	VH (120 residues)
Hu->Mu: Framework	0/112	9/120
CDR1	5/16	6/7
CDR2	3/7	10/16
CDR3	6/8	8/11
Total Hu->Mu	14/112 (12.5%)	33/120 (27.5%)
Mu->Hu: Framework	11/112	26/120
Backmutation notes	none	<ol style="list-style-type: none"> 1. Canonical: A24F, F29L, R71K 2. Packing: V37I 3. Vernier: T28S, V48L, F67L, N73T, L78V
Acceptor notes	<ol style="list-style-type: none"> 4. Genbank Acc. no. BAC01733 5. CDRs from same canonical structural group as donor mouse; 6. Immunoglobulin kappa light chain K64(AIMS4) 	<ol style="list-style-type: none"> 7. Genbank Acc. no. AAA69734 (H1 – class 1, H2 = class 3) 8. CDRs from same canonical structural group as donor mouse 9. fetal Ig

Tables 8 and 9 set forth Kabat numbering keys for the various light and heavy chains, respectively.

Table 8: Key to Kabat Numbering for 12A11 Light Chain

<u>KAB</u>	<u>#</u>	<u>TYPE</u>	<u>mouse</u> <u>12A11</u> <u>VL</u>	<u>HUM</u> <u>12A11</u> <u>VL</u>	<u>BAC</u> <u>01733</u>	<u>A19-</u> <u>Germ-</u> <u>line</u>	<u>Comment</u>
1	1	FR1	D	D	D	D	
2	2		V	V	V	I	<i>canonical</i>
3	3		L	V	V	V	
4	4		M	M	M	M	<i>vernier</i>
5	5		T	T	T	T	
6	6		Q	Q	Q	Q	
7	7		T	S	S	S	
8	8		P	P	P	P	
9	9		L	L	L	L	
10	10		S	S	S	S	
11	11		L	L	L	L	
12	12		P	P	P	P	
13	13		V	V	V	V	
14	14		S	T	T	T	
15	15		L	P	P	P	
16	16		G	G	G	G	
17	17		D	E	E	E	
18	18		Q	P	P	P	
19	19		A	A	A	A	
20	20		S	S	S	S	
21	21		I	I	I	I	
22	22		S	S	S	S	
23	23		C	C	C	C	

24	24	CDR1	R	R	R	R
25	25		S	S	S	S
26	26		S	S	S	S
27	27		Q	Q	Q	Q
27A	28		S	S	S	S
27B	29		I	I	L	L
27C	30		V	V	L	L
27D	31		H	H	H	H
27E	32		S	S	S	S
28	33		N	N	N	N
29	34		G	G	G	G
30	35		N	N	Y	Y
31	36		T	T	N	N
32	37		Y	Y	Y	Y
33	38		L	L	L	L
34	39		E	E	D	D
35	40	FR2	W	W	W	W
36	41		Y	Y	Y	Y
37	42		L	L	L	L
38	43		Q	Q	Q	Q
39	44		K	K	K	K
40	45		P	P	P	P
41	46		G	G	G	G
42	47		Q	Q	Q	Q
43	48		S	S	S	S
44	49		P	P	P	P
45	50		K	Q	Q	Q
46	51		L	L	L	L
47	52		L	L	L	L
48	53		I	I	I	I
49	54		Y	Y	Y	Y
50	55	CDR2	K	K	L	L
51	56		V	V	G	G
52	57		S	S	S	S
53	58		N	N	N	N
54	59		R	R	R	R
55	60		F	F	A	A
56	61		S	S	S	S

57	62	FR3	G	G	G	
58	63		V	V	V	
59	64		P	P	P	
60	65		D	D	D	
61	66		R	R	R	
62	67		F	F	F	
63	68		S	S	S	
64	69		G	G	G	<i>canonical</i>
65	70		S	S	S	
66	71		G	G	G	<i>vernier</i>
67	72		S	S	S	
68	73		G	G	G	<i>vernier</i>
69	74		T	T	T	<i>vernier</i>
70	75		D	D	D	
71	76		F	F	F	<i>canonical</i>
72	77		T	T	T	
73	78		L	L	L	
74	79		K	K	K	
75	80		I	I	I	
76	81		S	S	S	
77	82		R	R	R	
78	83		V	V	V	
79	84		E	E	E	
80	85		A	A	A	
81	86		E	E	E	
82	87		D	D	D	
83	88		L	V	V	
84	89		G	G	G	
85	90		I	V	V	
86	91		Y	Y	Y	
87	92		Y	Y	Y	<i>packing</i>
88	93		C	C	C	
89	94	CDR3	F	F	M	M
90	95		Q	Q	Q	Q
91	96		S	S	A	A
92	97		S	S	L	L
93	98		H	H	Q	Q
94	99		V	V	T	T
95	100		P	P	P	P
96	101		L	L	Y	
97	102		T	T	T	

98	103	FR4	F	F	F	<i>packing</i>
99	104		G	G	G	
100	105		A	Q	Q	
101	106		G	G	G	
102	107		T	T	T	
103	108		K	K	K	
104	109		L	L	L	
105	110		E	E	E	
106	111		L	I	I	
106A	112		K	K	K	

Table 9. Key to Kabat Numbering for 12A11 Heavy Chain

<u>KAB</u>	<u>TYPE</u>	<u>Mouse</u>	<u>HUM</u>	<u>567123</u>	<u>Germ-line</u>	<u>Comment</u>
#	#	<u>12A11</u> VH	<u>12A11</u> VHv1	<u>AAA</u> 69734		
1	1	FR1	Q	Q	Q	
2	2		V	V	V	<i>vernier</i>
3	3		T	Q	Q	
4	4		L	L	L	
5	5		K	V	V	
6	6		E	E	E	
7	7		S	S	S	
8	8		G	G	G	
9	9		P	G	G	
10	10		G	G	G	
11	11		I	V	V	V
12	12		L	V	V	V
13	13		K	Q	Q	
14	14		P	P	P	
15	15		S	G	G	
16	16		Q	R	R	
17	17		T	S	S	S
18	18		L	L	L	
19	19		S	R	R	
20	20		L	L	L	
21	21		T	S	S	
22	22		C	C	C	
23	23		S	A	A	
24	24		F	F	A	canonical for H1 - backmutate in v1
25	25		S	S	S	
26	26		G	G	G	<i>canonical</i>
27	27		F	F	F	<i>canonical</i>
28	28		S	S	T	<i>vernier</i> , close to H1 - backmutate in v1
29	29		L	L	F	canonical for H1 - backmutate in v1
30	30		S	S	S	

31	31	CDR1	T	T	S	S
32	32		S	S	Y	Y
33	33		G	G	A	A
34	34		M	M	M	M
35	35		S	S	H	H
35A	36		V	V	-	-
35B	37		G	G	-	-
36	38	FR2	W	W	W	W
37	39		I	I	V	V
38	40		R	R	R	R
39	41		Q	Q	Q	Q
40	42		P	A	A	A
41	43		S	P	P	P
42	44		G	G	G	G
43	45		K	K	K	K
44	46		G	G	G	G
45	47		L	L	L	L
46	48		E	E	E	E
47	49		W	W	W	W
48	50		L	L	V	V
49	51		A	A	A	A
50	52	CDR2	H	H	V	V
51	53		I	I	I	I
52	54		W	W	S	S
53	55		W	W	Y	Y
54	56		D	D	D	D
55	57		D	D	G	G
56	58		-	-	S	S
57	59		D	D	N	N
58	60		K	K	K	K
59	61		Y	Y	Y	Y
60	62		Y	Y	Y	Y
61	63		N	N	A	A
62	64		P	P	D	D
63	65		S	S	S	S
64	66		L	L	V	V
65	67		K	K	K	G
			S	S	G	

66	68	FR3	R	R	R	R	
67	69		L	L	F	F	vernier (underneath H2, possibly interacting with L63) - backmutate in v1
68	70		T	T	T	T	
69	71		I	I	I	I	
70	72		S	S	S	S	
71	73		K	K'	R	R	canonical for H2 - backmutate in v1
72	74		D	D	D	D	
73	75		T	T	N	N	vernier (edge of binding site, possibly interacting with S30) - backmutate in v1
74	76		S	S	S	S	
75	77		R	K	K	K	
76	78		N	N	N	N	
77	79		Q	T	T	T	
78	80		V	V	L	L	vernier (buried under H1, possibly interacting with V35A) - backmutate in v1
79	81		F	Y	Y	Y	
80	82		L	L	L	L	
81	83		K	Q	Q	Q	
82	84		I	M	M	M	
82A	85		T	N	N	N	
82B	86		S	S	S	S	
82C	87		V	L	L	L	
83	88		D	R	R	R	
84	89		T	A	A	A	
85	90		A	E	E	E	
86	91		D	D	D	D	
87	92		T	T	T	T	
88	93		A	A	A	A	
89	94		T	V	V	V	
90	95		Y	Y	Y	Y	
91	96		Y	Y	Y	Y	<i>packing</i>
92	97		C	C	C	C	
93	98		A	A	A	A	<i>packing canonical</i>
94	99		R	R	R	R	

			-	-	D	D
95	100	CDR3	R	R	R	-
96	101		T	T	H	-
97	102		T	T	S	-
98	103		T	T	S	-
99	104		A	A	S	A
100	105		D	D	W	K
100A	106		Y	Y	Y	L
100B	107		F	F	Y	L
101	108		A	A	G	M
102	109		Y	Y	M	L
			-	-	D	L
			-	-	V	I
103	110		W	W	W	<i>packing</i>
104	111		G	G	G	G
105	112		Q	Q	Q	A
106	113		G	G	G	K
107	114	FR4	T	T	T	G
108	115		T	T	T	Q
109	116		L	V	V	W
110	117		T	T	T	S
111	118		V	V	V	P
112	119		S	S	S	S
113	120		S	S	S	L

The humanized antibodies preferably exhibit a specific binding affinity for A β of at least 10^7 , 10^8 , 10^9 or 10^{10} M $^{-1}$. Usually the upper limit of binding affinity of the humanized antibodies for A β is within a factor of three, four or five of that of 12A11 (i.e., $\sim 10^9$ M $^{-1}$). Often the lower limit of binding affinity is also within a factor of three, four or five of that of 12A11.

Assembly and Expression of Humanized 12A11 VH and VL, Version 1
PCR-mediated assembly was used to generate h12A11v1 using appropriate oligonucleotide primers. The nucleotide sequences of humanized 12A11VL (version 1) (SEQ ID NO:34) and 12A11VH (version 1) (SEQ ID NO: 35) are listed below in Tables 10 and 11, respectively.

Table 10. Nucleotide sequence of humanized 12A11VLv1.

15 atgaggctccctgctcagctctggggctgtatgcgtctgggtctctggctccagtgggGATGTTGTGAT
GACCCAATCTCCACTCTCCCTGCCTGTCACTCCTGGAGAGGCCAGCCTCCATCTCTGCAGATCTAGTCAGA
GCATTGTGCATAGTAATGGAAACACCTACCTGGAATGGTACCTGCAGAACCCAGGCCAGTCTCCACAGCTC

CTGATCTACAAAGTTCCAACCGATTTCTGGGTCCCAGACAGGTCAGTGGCAGTGGATCAGGGACAGA
TTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGAGTTATTACTGCTTCAGGGACAGCTGGAGATCAA
(SEQ ID NO:34)

- 5 (uppercase VL segment only) leader peptide encoded by A19 germline seq derived from x63397 leader peptide

Table 11. Nucleotide sequence of humanized 12A11VHv1

10 atggagttgggctgagctgggtttcctcggttgcgttcgtggatgtccagggtgtcaagttcagctgggt
GGAGCTGGCGGGGGTGGTGCAGCCGGACGGTCCCCCAGGCTGTCTGTGCTTCTGGGTTTCAC
TGAGCACCTCTGGTATGAGTGTGGGCTGGATTCTCAGGCTCCAGGGAAGGGCTGGAGTGGCTGGCACAC
ATTTGGTGGGATGATGATAAGTACTATAACCCATCCCTGAAGAGCCGGCTCACAACTCCAAGGATAACCTC
CAAAAACACCGTGTACCTCCAGATGAACAGTCTGCCGCTGAAGATACTGCCGTGTACTACTGTGCTCGAA
GAACTACTACCGCTGACTACTTGCCTACTGGGCCAAGGCACCAGTGTACAGTCTCCTCA (SEQ ID
NO:35)

15 Leader peptide (lower case) derived from VH donor seq accession
M34030.1/aaa69734/M72

20 The amino acid sequences of humanized 12A11VL (version 1) (SEQ ID NO:7) and 12A11VH (version 1) (SEQ ID NO:10) are depicted in Figures 5A and 5B,
respectively.

B. Humanized 12A11 Antibodies - Versions 2, 2.1 and 3

The vernier residues (*e.g.*, S28T, V48L, F67L, L78V) contribute indirectly to CDR conformation and were postulated to be of least significance for conformational perturbation. The targeted residues were mutated by site-directed mutagenesis using a kit by Strategene and h12A11VHv1 in a pCRS plasmid as the mutagenesis template to arise at clones corresponding to version 2. A sequenced verified V-region insert of version 2 was subcloned into the BamHI/HindIII sites of the heavy chain expression vector pCMV-Cgamma1 to produce recombinant h12A11v2 antibody. A version 2.1 antibody was similarly created having each of the above vernier residue mutations (*i.e.*, elimination of backmutations) in addition to mutation at position T73N. A version 3 antibody likewise had each of the above mutations, T28S, L48V, L67F, V78L, in addition to a mutation at position K71R.

C. Humanized 12A11 Antibodies - Versions 4 to 6

35 Additional humanized 12A11 versions were designed which retained backmutations at canonical and packing residues but eliminated backmutations at one (versions 4.1 to 4.4), two (versions 5.1 to 5.6) or three (versions 6.1 to 6.4) vernier residues. Site-directed mutagenesis and clone construction was performed as described in subpart C, above. Recombinant antibodies were expressed in COS cells and purified

from COS cell supernatants. Additional versions may include combinations of the above, for example, human residues at 1, 2, 3, 4 or 5 vernier residues in combination with at least one packing and/or canonical residue (*e.g.*, human residues at positions 28, 37, 48, 67, 71 and 78 or human residues at positions 28, 37, 48, 67, 71, 73 and 78).

5

D. Humanized 12A11 Antibodies - Versions 7 and 8

A seventh version of humanized 12A11 is created having each of the backmutations indicated for version 1, except for the T→S backmutation at residue 28 (vernier), and the V→I backmutation at residue 37 (packing). An eighth version of 10 humanized 12A11 is created having each of the backmutations indicated for version 1, except for the N→T backmutation at residue 73 (vernier). The amino acid sequences of humanized 12A11 version 7 and 8 heavy chains are set forth as SEQ ID NOs: 30 and 31 respectively.

As compared to version 1, version 7 contains only 7 backmutations. The 15 T28S backmutation is conservative and is eliminated in version 7 of the heavy chain. The backmutation at packing residue V37I is also eliminated in version 7. As compared to version 1, version 7 contains only 8 backmutations. In version 8, the N73T (vernier) backmutation is eliminated.

Additional versions may include combinations of the above, for example, 20 human residues (*e.g.*, elimination of backmutations) at 1, 2, 3, 4 (or 5) residues selected from positions 28, 48, 78 and 73, optionally in combination with elimination of backmutation at at least one packing residue (*e.g.*, position 37) and/or at least one canonical residue.

25 **Example VI: Functional Texting of Humanized 12A11 Antibodies**

Humanized 12A11 version 1 was cloned as described in Example V. Humanized 12A11 was produced by transient expression in COS cells, and purified according to art-recognized methodologies. The binding activity of the humanized antibody was first demonstrated by a qualitative ELISA assay (data not shown). 30 Humanized 12A11 version 1 was further compared to its murine and chimeric counterparts for two properties: antigen binding (quantitative A β ELISA) and relative affinity. The binding activity of h12A11v1 was demonstrated in the quantitative A β

ELISA and found to be identical with murine and chimeric forms of 12A11 (see Figure 7).

The affinity of h12A11v1 antibody was also compared with murine and chimeric 12A11 antibodies by a competitive A β ELISA. For the competitive binding assay, a biotin conjugated recombinant mouse 12A11C γ 2a (isotype switched 12A11) was used. The binding activity of the biotinylated m12A11 C γ 2a for aggregate A β 1-42 was confirmed by an ELISA assay using streptavidin-HRP as reporter. A direct comparison of A β binding by the two isoforms of 12A11 (C γ 1, C γ 2a), using HRP conjugated goat anti-mouse HRP as reporter, confirmed that the biotin conjugated recombinant 12A11C γ 2a is comparable to the original C γ 1 mouse antibody.

Competition binding study employed the biotin conjugated m12A11 C γ 2a at a fixed concentration and competing with a range of concentrations of test antibodies as indicated in Figure 8. Figure 8 shows the result of h12A11v1 competitive assay comparing h12A11v1 with chimeric and murine forms. The humanized 12A11v1 competed within 2X IC₅₀ value with its murine and chimeric counterparts. This data is consistent with affinity determination using Biacore technology (data not shown), which indicated KD values of 38nM and 23 nM for the murine C γ 2a and h12A11v1, respectively. In summary, the findings suggest h12A11v1 retains the antigen binding properties and affinity of its original murine counterpart.

COS cells were transiently transfected with different combinations of humanized 12A11VH and h12A11VLv1. The conditioned media was collected 72 hours post-transfection. Antibody concentration in conditioned media from transfected COS cells was determined by a quantitative human IgG ELISA. Quantitative A β (1-42) aggregate binding assay confirmed that h12A11v2, v2.1 and v3 are comparable to h12A11v1 and to chimeric 12A11 for antigen binding. Moreover, versions 5.1-5.6 and 6.1-6.3 exhibit similar binding activities when tested in this binding assay. Version 6.4 showed some loss of activity in the assay but activity was notably restored in v2.

Binding properties for murine 12A11 and h12A11v1 were also compared using BIACore technology. Murine 12A11 and h12A11v1 exhibited similar binding profiles when exposed to either low- or high-density immobilized A β peptide (bio-DAE peptide). Kinetic analysis of murine 12A11 versus h12A11v1 was also performed. In these studies the BIACore technology was used to measure the binding of soluble antibody to solid phase bound biotinylated DAE peptide. The peptide was immobilized

on streptavidin biosensor chips then, varying concentrations of each antibody were applied in triplicates and the binding was measured as a function of time. The kinetic data was analyzed using BIAevaluation software applied to a bivalent model. The apparent dissociation (k_d) and association (k_a) rate constants were calculated from the appropriate regions of the sensorgrams using a global analysis. The affinity constant of the interaction between bio-DAE10 and the antibodies was calculated from the kinetic rate constants. From these measurements the apparent dissociation (k_d) and association (k_a) rate constants were derived and used to calculate a K_D value for the interaction.

Table 12 includes a summary of kinetic analysis of $A\beta$ binding of 12A11 antibodies as determined by BIACore analysis.

Table 12:

Bivalent Model (global analysis)					
Antibody	K_a (1/Ms)	K_d (1/s)	K_A (1/M)	K_D (nM)	Chi2
m12A11	1.05E+05	3.98E-03	2.64E+07	38.00	0.247
h12A11v1	1.47E+05	3.43E-03	4.29E+07	23.30	0.145

The data indicate that humanized 12A11 v1 has a similar affinity for $A\beta$ peptide when compare with parental murine 12A11.

Example VII. Prevention and Treatment of Human Subjects

A single-dose phase I trial is performed to determine safety in humans. A therapeutic agent is administered in increasing dosages to different patients starting from about 0.01 the level of presumed efficacy, and increasing by a factor of three until a level of about 10 times the effective mouse dosage is reached.

A phase II trial is performed to determine therapeutic efficacy. Patients with early to mid Alzheimer's Disease defined using Alzheimer's disease and Related Disorders Association (ADRDA) criteria for probable AD are selected. Suitable patients score in the 12-26 range on the Mini-Mental State Exam (MMSE). Other selection criteria are that patients are likely to survive the duration of the study and lack complicating issues such as use of concomitant medications that may interfere. Baseline evaluations of patient function are made using classic psychometric measures, such as the MMSE, and the ADAS, which is a comprehensive scale for evaluating patients with

Alzheimer's Disease status and function. These psychometric scales provide a measure of progression of the Alzheimer's condition. Suitable qualitative life scales can also be used to monitor treatment. Disease progression can also be monitored by MRI. Blood profiles of patients can also be monitored including assays of immunogen-specific antibodies and T-cells responses.

5 Following baseline measurements, patients begin receiving treatment. They are randomized and treated with either therapeutic agent or placebo in a blinded fashion. Patients are monitored at least every six months. Efficacy is determined by a significant reduction in progression of a treatment group relative to a placebo group.

10 A second phase II trial is performed to evaluate conversion of patients from non-Alzheimer's Disease early memory loss, sometimes referred to as age-associated memory impairment (AAMI) or mild cognitive impairment (MCI), to probable Alzheimer's disease as defined as by ADRDA criteria. Patients with high risk for conversion to Alzheimer's Disease are selected from a non-clinical population by

15 screening reference populations for early signs of memory loss or other difficulties associated with pre-Alzheimer's symptomatology, a family history of Alzheimer's Disease, genetic risk factors, age, sex, and other features found to predict high-risk for Alzheimer's Disease. Baseline scores on suitable metrics including the MMSE and the ADAS together with other metrics designed to evaluate a more normal population are

20 collected. These patient populations are divided into suitable groups with placebo comparison against dosing alternatives with the agent. These patient populations are followed at intervals of about six months, and the endpoint for each patient is whether or not he or she converts to probable Alzheimer's Disease as defined by ADRDA criteria at the end of the observation.

25

Although the foregoing invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims. All publications and patent documents cited herein, as well as text appearing in the figures and sequence listing, are hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.

From the foregoing it will be apparent that the invention provides for a number of uses. For example, the invention provides for the use of any of the antibodies to A β described above in the treatment, prophylaxis or diagnosis of amyloidogenic disease, or in the manufacture of a medicament or diagnostic composition for use in the
5 same.

We claim:

1. A humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 12A11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence.
5
2. A humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 12A11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 12A11 light chain variable region sequence, wherein the framework residue is selected from the group consisting of:
10
15 (a) a residue that non-covalently binds antigen directly;
(b) a residue adjacent to a CDR;
(c) a CDR-interacting residue; and
(d) a residue participating in the VL-VH interface.
3. A humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 12A11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain
20
4. A humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 12A11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 12A11 heavy chain variable region sequence, wherein the framework residue is selected from the group consisting of:
25
30
16 (a) a residue that non-covalently binds antigen directly;
(b) a residue adjacent to a CDR;

- (c) a CDR-interacting residue; and
- (d) a residue participating in the VL-VH interface.

5. The light chain of claim 2, wherein a CDR-interacting residue is
5 identified by modeling the 12A11 light chain based on the solved structure of a murine
immunoglobulin light chain that shares at least 70% sequence identity with the 12A11
light chain.

6. The light chain of claim 2, wherein a CDR-interacting residue is
10 identified by modeling the 12A11 light chain based on the solved structure of a murine
immunoglobulin light chain that shares at least 80% sequence identity with the 12A11
light chain.

7. The light chain of claim 2, wherein a CDR-interacting residue is
15 identified by modeling the 12A11 light chain based on the solved structure of a murine
immunoglobulin light chain that shares at least 90% sequence identity with the 12A11
light chain.

8. The heavy chain of claim 4, wherein a CDR-interacting residue is
20 identified by modeling the 12A11 heavy chain based on the solved structure of a murine
immunoglobulin heavy chain that shares at least 70% sequence identity with the 12A11
heavy chain.

9. The heavy chain of claim 4, wherein a CDR-interacting residue is
25 identified by modeling the 12A11 heavy chain based on the solved structure of a murine
immunoglobulin heavy chain that shares at least 80% sequence identity with the 12A11
heavy chain.

10. The heavy chain of claim 4, wherein a CDR-interacting residue is
30 identified by modeling the 12A11 heavy chain based on the solved structure of a murine
immunoglobulin heavy chain that shares at least 90% sequence identity with the 12A11
heavy chain.

11. A humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 12A11 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 12A11 light chain variable region sequence, wherein the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the 12A11 immunoglobulin light chain variable region.

10

12. A humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 12A11 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 12A11 heavy chain variable region sequence, wherein the framework residue is a residue capable of affecting heavy chain variable region conformation or function as identified by analysis of a three-dimensional model of the 12A11 immunoglobulin heavy chain variable region.

15

13. The light chain of claim 11, wherein the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, a rare residue, and a glycosylation site residue on the surface of the structural model.

20

14. The heavy chain of claim 12, wherein the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, and a glycosylation site residue on the surface of the structural model.

15. The light chain of claim 11, wherein the framework residue is substituted in at least one position selected from the group consisting of position 2, 4, 36, 38, 40, 44, 46, 47, 48, 49, 64, 66, 68, 69, 71, 87 and 98 of the light chain as numbered according to
5 Kabat.

16. The heavy chain of claim 12, wherein the framework residue is substituted in at least one position selected from the group consisting of position 2, 24, 26, 27, 28, 29, 37, 39, 45, 47, 48, 67, 71, 73, 78, 91, 93, 94 and 103 of the heavy chain as
10 numbered according to Kabat.

17. The heavy chain of claim 12, wherein the framework residue is substituted at a position selected from the group consisting of position 24, 28, 29, 37, 48, 67, 71, 73 and 78 of the heavy chain as numbered according to Kabat.

15
18. The light chain of claim 11 or 13, wherein the framework residue is identified by modeling the 12A11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 70% sequence identity with the 12A11 light chain.

20
19. The light chain of claim 11 or 13, wherein the framework residue is identified by modeling the 12A11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 80% sequence identity with the 12A11 light chain.

25
20. The light chain of claim 11 or 13, wherein the framework residue is identified by modeling the 12A11 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 90% sequence identity with the 12A11 light chain.

30

21. The heavy chain of claim 12 or 14, wherein the framework residue is identified by modeling the 12A11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 70% sequence identity with the 12A11 heavy chain.

5

22. The heavy chain of claim 12 or 14, wherein the framework residue is identified by modeling the 12A11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 80% sequence identity with the 12A11 heavy chain.

10

23. The heavy chain of claim 12 or 14, wherein the framework residue is identified by modeling the 12A11 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 90% sequence identity with the 12A11 heavy chain.

15

24. A humanized immunoglobulin comprising the light chain of any one of claims 1, 2, 5-7, 11, 13, 15 and 18-20, and the heavy chain of any one of claims 3, 4, 8-10, 12, 14, 16-17 and 21-23, or antigen-binding fragment of said immunoglobulin.

20

25. The immunoglobulin or antigen binding fragment of claim 24, which specifically binds to beta amyloid peptide (A β) with a binding affinity of at least 10^{-7} M.

26. The immunoglobulin or antigen binding fragment of claim 24, which specifically binds to beta amyloid peptide (A β) with a binding affinity of at least 10^{-8} M.

25

27. The immunoglobulin or antigen binding fragment of claim 24, which specifically binds to beta amyloid peptide (A β) with a binding affinity of at least 10^{-9} M.

30

28. The immunoglobulin or antigen binding fragment of claim 24, wherein the heavy chain isotype is $\gamma 1$.

29. The immunoglobulin or antigen binding fragment of claim 24, which binds to soluble beta amyloid peptide (A β).

30. The immunoglobulin or antigen binding fragment of claim 24, which binds to aggregated beta amyloid peptide (A β).

5 31. The immunoglobulin or antigen binding fragment of claim 24, which mediates phagocytosis of beta amyloid peptide (A β).

32. The immunoglobulin or antigen binding fragment of claim 24, which crosses the blood-brain barrier in a subject.

10 33. The immunoglobulin or antigen binding fragment of claim 24, which reduces beta amyloid peptide (A β) plaque burden in a subject.

15 34. A humanized immunoglobulin comprising a humanized heavy chain and a humanized light chain, wherein

20 (a) the humanized light chain comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarily determining regions of the mouse 12A11 immunoglobulin light chain variable domain designated SEQ ID NO:2, and a variable region framework from a human light chain variable region framework sequence provided that at least one framework residue selected from the group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue, is occupied by the same amino acid residue present in the 25 equivalent position of the mouse 12A11 immunoglobulin light chain variable region framework; and

30 (b) the humanized heavy chain comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the mouse 12A11 immunoglobulin heavy chain variable domain designated SEQ ID NO:4, and a variable region framework from a human heavy chain variable region framework sequence provided that at least one framework residue selected from a second group consisting of a canonical residue, a vernier residue, a packing

residue and a rare residue, is occupied by the same amino acid residue present in the equivalent position of the mouse 12A11 immunoglobulin heavy chain variable region framework;

5 wherein the humanized immunoglobulin specifically binds to beta amyloid peptide ("A β ") with a binding affinity of at least 10^{-7} M, wherein the 12A11 immunoglobulin has the light chain with a variable domain designated SEQ ID NO:2 and the heavy chain with a variable domain designated SEQ ID NO:4.

10 35. The humanized immunoglobulin of claim 34, wherein human light chain variable region framework is from a kappa light chain variable region.

36. The humanized immunoglobulin of claim 34, wherein human heavy chain variable region framework is from an IgG1 heavy chain variable region.

15 37. The humanized immunoglobulin of claim 34, wherein the light chain variable region framework is from a human immunoglobulin light chain having at least 70% sequence identity with light chain sequence of the 12A11 immunoglobulin.

20 38. The humanized immunoglobulin of claim 34, wherein the heavy chain variable region framework is from a human immunoglobulin heavy chain having at least 70% sequence identity with heavy chain sequence of the 12A11 immunoglobulin.

25 39. The humanized immunoglobulin of claim 34, wherein the humanized light chain comprises complementarity determining regions that are identical to the corresponding complementarity determining regions of the mouse 12A11 heavy chain, and the humanized heavy chain comprises complementarity determining regions that are identical to the corresponding complementarity determining regions of the mouse 12A11 heavy chain.

30 40. A humanized antibody comprising the complementarity determining regions (CDR1, CDR2 and CDR3) of the 12A11 variable light chain sequence set forth as SEQ ID NO:2.

41. A humanized antibody comprising the complementarity determining regions (CDR1, CDR2 and CDR3) of the 12A11 variable heavy chain sequence set forth as SEQ ID NO:4.

5

42. A humanized antibody, or antigen-binding fragment thereof, which specifically binds to beta amyloid peptide (A β), comprising a variable region comprising complementarity determining regions (CDRs) corresponding to CDRs from the mouse 12A11 antibody.

10

43. A humanized immunoglobulin having the variable light chain amino acid sequence set forth as SEQ ID NO:6 and having a variable heavy chain amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

15

44. A chimeric immunoglobulin comprising variable region sequence substantially as set forth in SEQ ID NO:2 or SEQ ID NO:4, and constant region sequences from a human immunoglobulin.

20

45. A method of preventing or treating an amyloidogenic disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of claims 24-43.

25

46. A method of preventing or treating Alzheimer's disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of claims 24-43.

47. The method of claim 46, wherein the effective dosage of humanized immunoglobulin is at least about 1 mg/kg body weight.

30

48. The method of claim 46, wherein the effective dosage of humanized immunoglobulin is at least about 10 mg/kg body weight.

49. A pharmaceutical composition comprising the immunoglobulin of any one of claims 24-43 and a pharmaceutical carrier.
50. An isolated polypeptide comprising a fragment of SEQ ID NO:2 selected from the group consisting of amino acids 43-58 of SEQ ID NO:2, amino acids 74-80 of SEQ ID NO:2 and amino acids 113-121 of SEQ ID NO:2.
51. An isolated polypeptide comprising amino acids 43-48 of SEQ ID NO:2, amino acids 74-80 of SEQ ID NO:2 and amino acids 113-121 of SEQ ID NO:2.
- 10 52. An isolated polypeptide comprising a fragment of SEQ ID NO:4 selected from the group consisting of amino acids 50-56 of SEQ ID NO:4, amino acids 71-86 of SEQ ID NO:4 and amino acids 119-128 of SEQ ID NO:4.
- 15 53. An isolated polypeptide comprising amino acids 50-56 of SEQ ID NO:4, amino acids 71-86 of SEQ ID NO:4 and amino acids 119-128 of SEQ ID NO:4.
54. An isolated polypeptide comprising amino acids 1-131 of SEQ ID NO:2.
- 20 55. An isolated polypeptide comprising amino acids 1-139 of SEQ ID NO:4.
56. An isolated polypeptide having at least 85% identity to amino acids 1-131 of SEQ ID NO: 2.
- 25 57. An isolated polypeptide having at least 85% identity to amino acids 1-139 of SEQ ID NO: 4.
58. The isolated polypeptide of claim 56 or 57, wherein the polypeptide has at least 86% identity.
- 30 59. The isolated polypeptide of claim 56 or 57, wherein the polypeptide has at least 87% identity.

60. The isolated polypeptide of claim 56 or 57, wherein the polypeptide has at least 88% identity.

61. The isolated polypeptide of claim 56 or 57, wherein the polypeptide has
5 at least 89% identity.

62. The isolated polypeptide of claim 56 or 57, wherein the polypeptide has at least 90% identity.

10 63. The isolated polypeptide of claim 56 or 57, wherein the polypeptide has at least 90% or more identity.

15 64. A variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, said variant comprising at least one conservative amino acid substitution, wherein the variant retains the ability to specifically bind beta amyloid peptide (A β) with a binding affinity of at least 10^7 M $^{-1}$.

20 65. A variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, said variant comprising at least one conservative amino acid substitution, wherein the variant retains the ability to direct specific binding to beta amyloid peptide (A β) with a binding affinity of at least 10^7 M $^{-1}$.

25 66. An isolated nucleic acid molecule encoding the light chain of any one of claims 1, 2, 5-7, 11, 13, 15 and 18-20.

67. An isolated nucleic acid molecule encoding the heavy chain of any one of claims 3, 4, 8-10, 12, 14, 16-17 and 21-23.

30 68. An isolated nucleic acid molecule encoding the polypeptide of any one of claims 46-65.

69. An isolated nucleic acid molecule encoding the immunoglobulin of any one of claims 21-44.

70. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 3.

5 71. A vector comprising the nucleic acid molecule of any of claims 66-70.

72. A host cell comprising the nucleic acid molecule of any of claims 66-70.

10 73. A transgenic animal expressing a polypeptide encoded by the nucleic acid molecule of any of the preceding claims.

74. The transgenic animal of claim 73, wherein the polypeptide is expressed in the milk of said animal.

15 75. A method of producing an antibody, or fragment thereof, comprising culturing the host cell of claim 72 under conditions such that the antibody or fragment is produced and isolating said antibody from the host cell or culture.

20 76. A method of producing an antibody or antibody fragment, comprising a fragment of SEQ ID NO:2 selected from the group consisting of amino acids 43-58 of SEQ ID NO:2, amino acids 74-80 of SEQ ID NO:2 and amino acids 113-121 of SEQ ID NO:2, said method comprising culturing a host cell comprising a nucleic acid molecule that encodes said antibody or antibody fragment under conditions such that the antibody or antibody fragment is produced, and isolating said antibody or antibody fragment from 25 the host cell or culture.

77. A method of producing an antibody or antibody fragment, comprising a fragment of SEQ ID NO:4 selected from the group consisting of amino acids 50-56 of SEQ ID NO:4, amino acids 71-86 of SEQ ID NO:4 and amino acids 119-128 of SEQ ID NO:4, said method comprising culturing a host cell comprising a nucleic acid molecule that encodes said antibody or antibody fragment, under conditions such that the antibody or antibody fragment is produced, and isolating said antibody or antibody fragment from the host cell or culture.

78. A method for identifying residues amenable to substitution in a humanized 12A11 immunoglobulin variable framework region, comprising modeling the three-dimensional structure of the 12A11 variable region based on a solved 5 immunoglobulin structure and analyzing said model for residues capable of affecting 12A11 immunoglobulin variable region conformation or function, such that residues amenable to substitution are identified.

79. Use of the variable region sequence set forth as SEQ ID NO:2 or SEQ ID 10 NO:4, or any portion thereof, in producing a three-dimensional image of a 12A11 immunoglobulin, 12A11 immunoglobulin chain, or domain thereof.

80. A methods of imaging amyloid deposits in the brain of a patient comprising administering to the patient an agent that specifically binds to A β , and 15 detecting the antibody bound to A β .

81. The method of claim 80, wherein the agent is an antibody comprising a light chain variable sequence as set forth in SEQ ID NO:2 and a heavy chain variable region sequence as set forth in SEQ ID NO:4, or an antigen-binding fragment of said 20 antibody.

82. The method of claim 80, wherein the antigen-binding fragment is a Fab fragment.

83. A kit for imaging according to the method of any one of claims 80-82 including instructions for use.

84. A method of treating an amyloidogenic disease comprising administering to a patient having said amyloidogenic disease, a nucleic acid molecule that encodes an 30 immunoglobulin light chain comprising the CDRs of the amino acid sequence of SEQ ID NO:2 and a nucleic acid molecule that encodes an immunoglobulin heavy chain that comprises the CDRs of the amino acid sequence of SEQ ID NO:4, under conditions such

that said immunoglobulin chains are expressed, such that a beneficial therapeutic response in said patient is generated.

5 85. A method of treating an amyloidogenic disease comprising administering to a patient having said amyloidogenic disease, a nucleic acid molecule that encodes an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:7 and a nucleic acid molecule that encodes an immunoglobulin heavy chain that comprises the amino acid sequence of SEQ ID NO:10 or the amino acid sequence of any one of SEQ
10 ID NOs: 13-31, under conditions such that said immunoglobulin chains are expressed, such that a beneficial therapeutic response in said patient is generated.

86. A method of preventing or treating a disease associated with amyloid deposits of A β in the brain of a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of the preceding claims.
15

87. The method of claim 86, wherein the disease is characterized by cognitive impairment.

20 88. The method of claim 86, wherein the disease is Alzheimer's disease.

89. The method of claim 86, wherein the disease is Down's syndrome.

90. The method of claim 86, wherein the disease is mild cognitive impairment.

25 91. The method of claim 86, wherein the antibody is of human isotype IgG1.

92. The method of any of the preceding claims, wherein the patient is human.

30 93. The method of any one of claims 86-92, wherein after administration the antibody binds to an amyloid deposit in the patient and induces a clearing response against the amyloid deposit.

94. The method of claim 93, wherein the clearing response is an Fc receptor-mediated phagocytosis response.

5 95. The method of claim 93 or 94, further comprising monitoring the clearing response.

96. The method of any one of claims 86-92, wherein after administration the antibody binds to soluble A β in the patient.

10 97. The method of any one of claims 86-92, wherein after administration the antibody binds to soluble A β in the serum, blood or cerebrospinal fluid of the patient.

15 98. The method of any one of claims 86-97, wherein the patient is asymptomatic.

99. The method of any one of claims 86-98, wherein the patient is under 50 years of age.

20 100. The method of any one of claims 86-99, wherein the patient has an inherited risk factor indicating susceptibility to Alzheimer's disease.

101. The method of any one of claims 86-100, wherein the dosage of antibody is at least 1 mg/kg body weight of the patient.

25 102. The method of any one of claims 86-100, wherein the dosage of antibody is at least 10 mg/kg body weight of the patient.

103. The method of any one of claims 86-102, wherein the antibody is administered with a carrier as a pharmaceutical composition.

30 104. The method of any one of claims 77-103, wherein the antibody is administered intraperitoneally, orally, intranasally, subcutaneously, intramuscularly, topically or intravenously.

105. A method for reducing plaque burden in a subject in need thereof comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of the preceding claims.

5

106. The method of claim 105, wherein after administration the antibody binds to an amyloid deposit in the patient and induces a clearing response against the amyloid deposit.

10 107. The method of claim 106, wherein the clearing response is an Fc receptor-mediated phagocytosis response.

108. The method of claim 106 or 107, further comprising monitoring the clearing response.

15

109. The method of claim 105, wherein after administration the antibody binds to soluble A β in the patient.

20 110. The method of claim 105, wherein after administration the antibody binds to soluble A β in the serum, blood or cerebrospinal fluid of the patient.

111. A humanized immunoglobulin of any one of the preceding claims, which comprises a Fc region having an altered effector function.

25

112. A method for reducing neuritic burden in a subject in need thereof comprising administering to the subject an effective dose of a humanized 12A11 antibody which binds to an epitope within amino acids 3-7 of beta amyloid peptide (A β), wherein the antibody is of the IgG1 isotype.

30

113. The method of claim 112, wherein the subject has an amyloidogenic disease.

114. The method of claim 113, wherein the amyloidogenic disease is Alzheimer's disease.

115. A method for treating an amyloidogenic disease in a patient comprising
5 administering to the patient an effective dose of a humanized 12A11 antibody capable of reducing beta amyloid peptide (A β) burden and neuritic dystrophy in the patient, wherein the antibody binds to an epitope within amino acids 3-7 of A β and is of the IgG1 isotype.

10 116. The method of claim 115, wherein the amyloidogenic disease is Alzheimer's disease.

117. A method for reducing beta amyloid peptide (A β) burden and neuritic dystrophy in a mammal comprising administering to the mammal an effective dose of a
15 humanized 12A11 antibody capable of reducing beta amyloid peptide (A β) burden and neuritic dystrophy, wherein the antibody binds to an epitope within amino acids 3-7 of A β and is of the IgG1 isotype.

118. The method of claim 117, wherein the mammal is a human.
20

119. The method of claim 117 or 118, wherein the mammal has an amyloidogenic disease.

25 120. The method of claim 119, wherein the amyloidogenic disease is Alzheimer's disease.

121. A therapeutic composition comprising a humanized 12A11 antibody of the IgG1 isotype which binds to an epitope within amino acids 3-7 of beta amyloid peptide (A β), wherein the antibody is capable of reducing neuritic burden in a subject.

30 122. A method for treating an amyloidogenic disease in a patient comprising administering to the patient an effective dose of a humanized 12A11 antibody which binds to soluble beta amyloid peptide (A β) and reduces neuritic dystrophy in the patient,

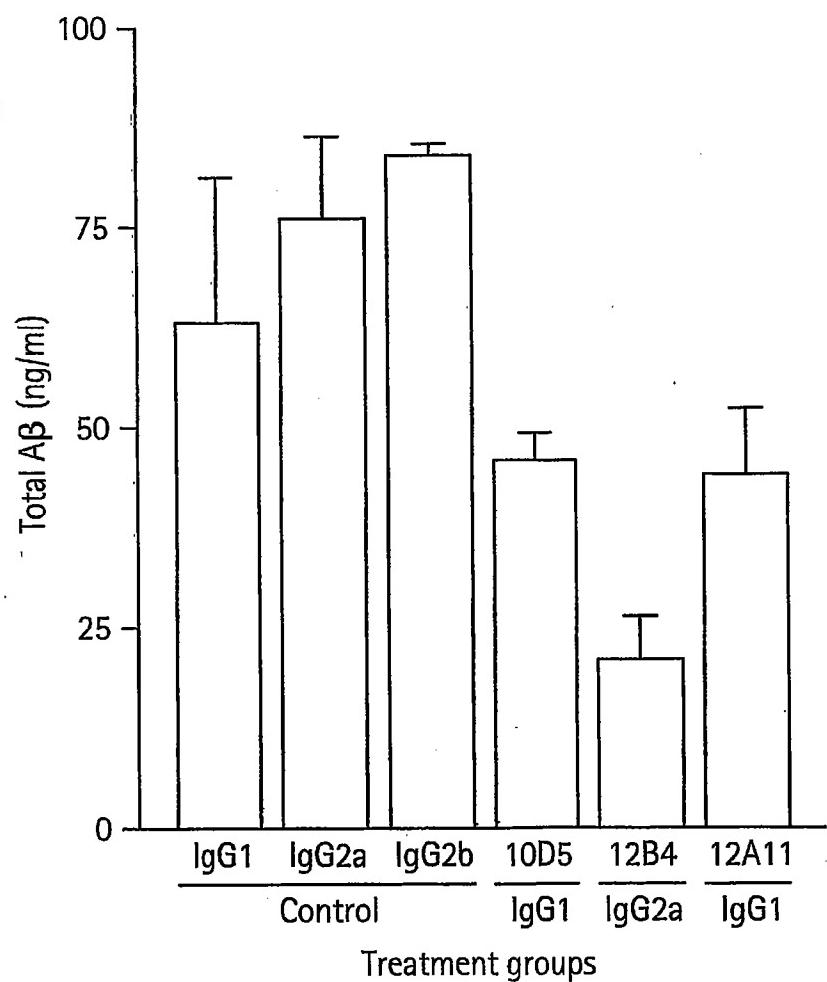
wherein the antibody binds to an epitope within amino acids 3-7 of A β and is of the IgG1 isotype.

123. The method of claim 122, wherein the amyloidogenic disease is
5 Alzheimer's disease.

124. A therapeutic composition comprising a humanized 12A11 antibody of the IgG1 isotype which binds to soluble beta amyloid peptide (A β), wherein the antibody binds to an epitope within amino acids 3-7 of A β and is capable of reducing neuritic
10 burden in a subject.

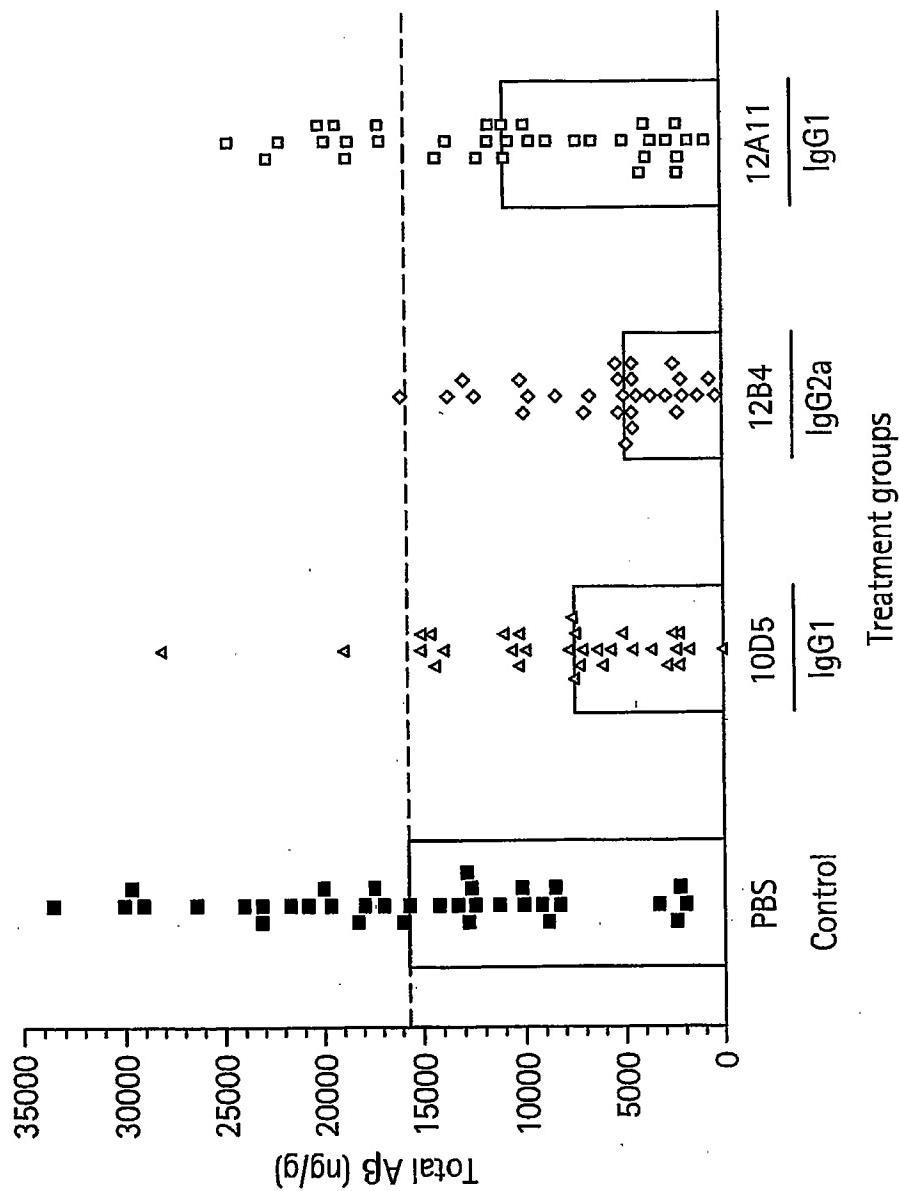
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FIG. 1



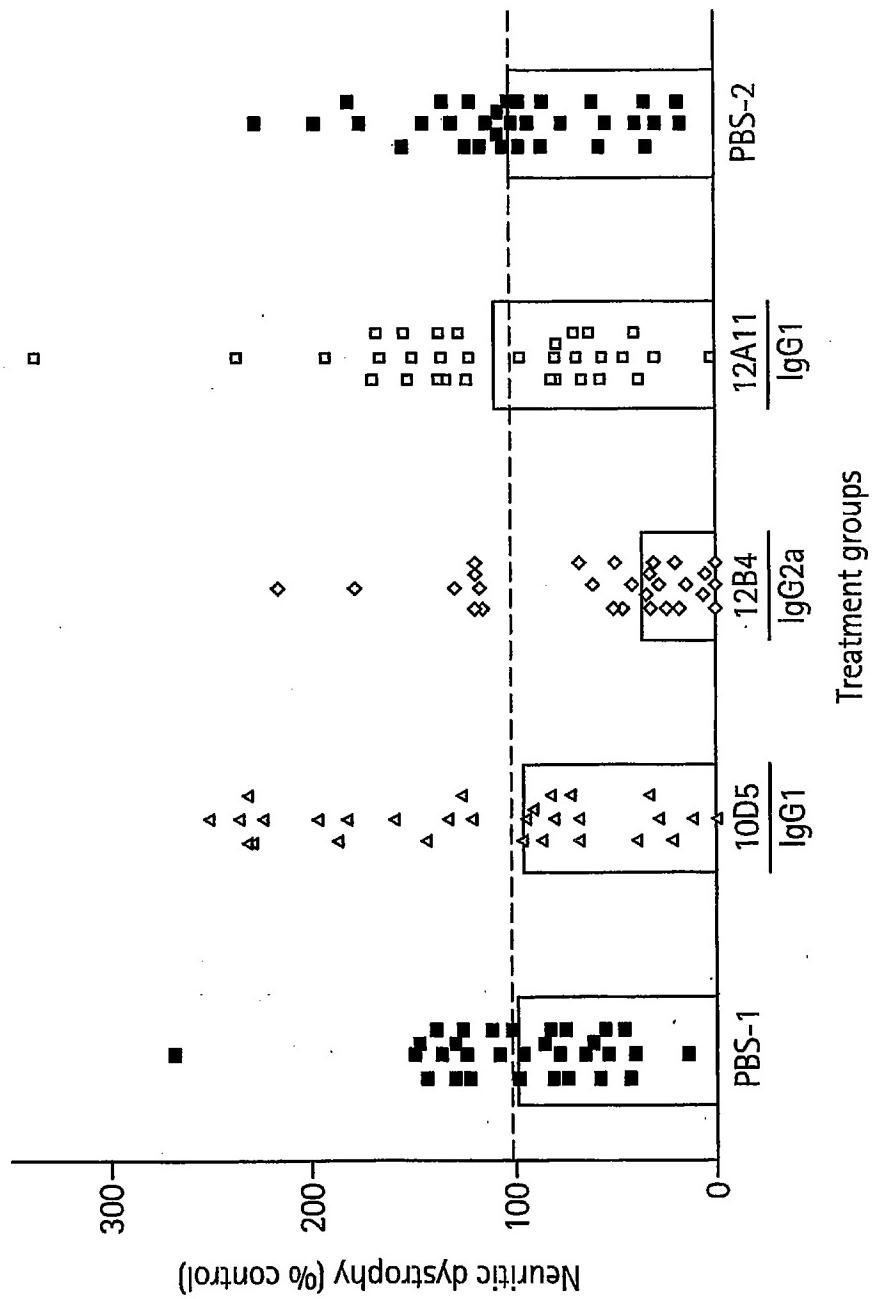
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FIG. 2A



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FIG. 2B



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FIG. 3A

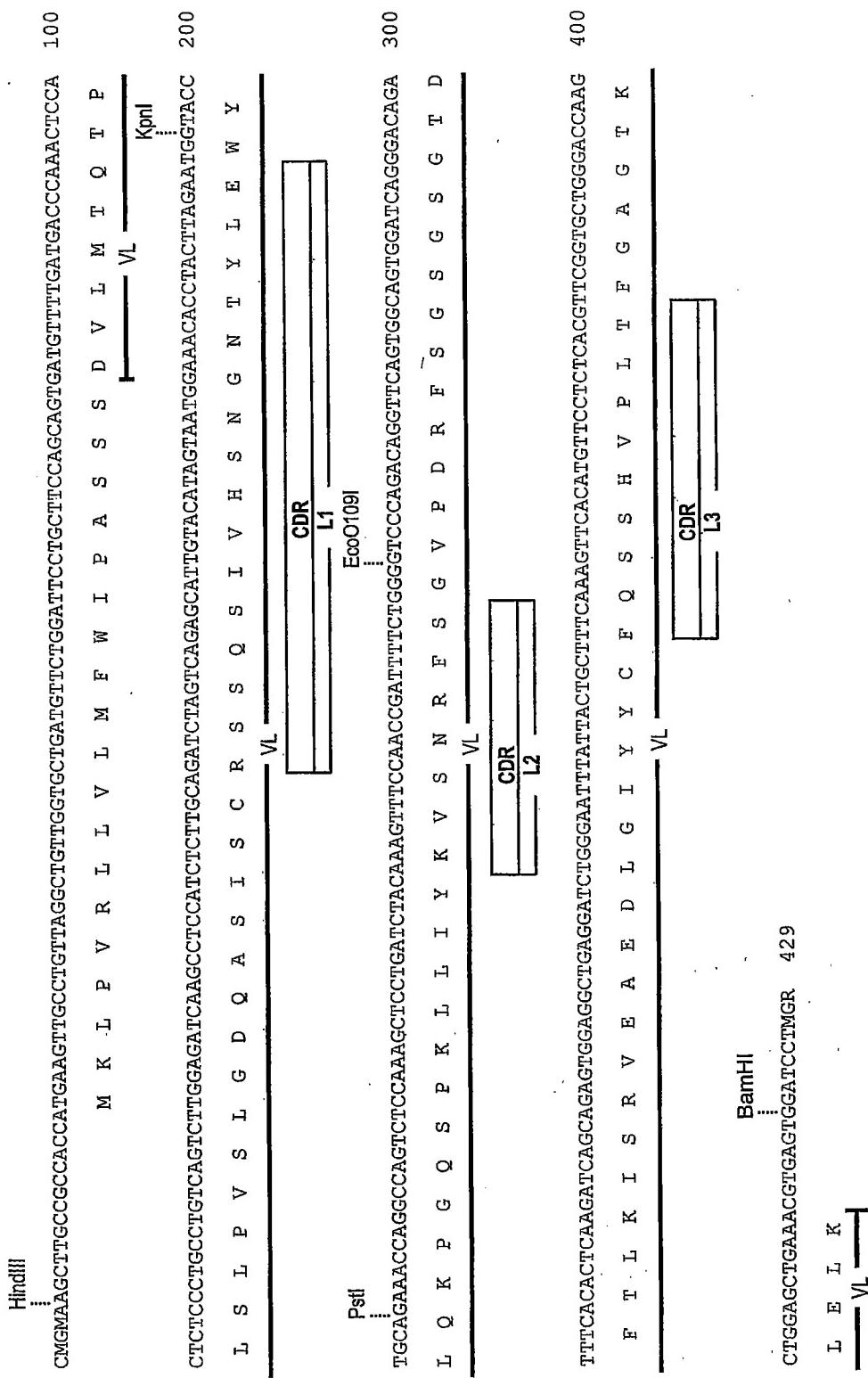


FIG. 3B

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VH
KpnI

GGATATTGAAGGCCCTCACAGACCCTCAGTCTGACTCTGTTTCACTGAGGACTCTGGTATGAGGTAGGGTAGGCTGGATTCAGCTTC 200

G I L K P S Q T L S L T C S F S G F S L S T S G M S V G W I R Q P S

VH

PstI

AGGGAAAGGGCTGGAGTGGCTGGCACACATTGGTGGATGATAAGTACTATAACCATAACCATCCCTGAAGAGGCCGGCTCACAACTCCAAAGGATAACCTCC 300

G K G L E W L A H I W W D D K Y Y N P S L K S R L T I S K D T S

VH

BamHI

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R N Q V F L K I T S V D T A D T A T Y Y C A R R T T T A D Y F A Y

VH

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W G Q G T T L T V S S

VH
H3

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FIG. 4

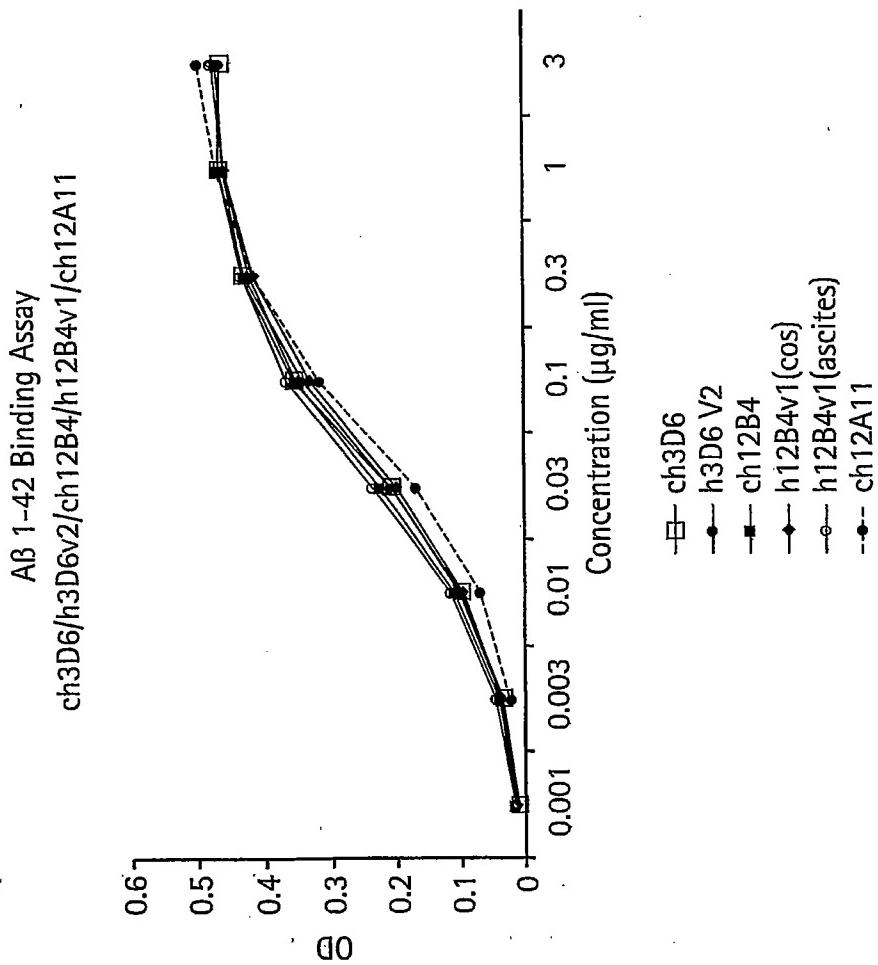


FIG. 5A

h12A11 VI Design

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bac01733	M K Y L L P T A A A G L L L A A Q P A M A	D V V M T Q S P L S L P V T P G E P	40									
A19 VL Vlk2-28	M R - - L P A Q I L G L L M L W V S G S S G	D T V M T Q S P L S L P V T P G E P	38									
Ch12A11VL	A S I S C R S S Q S I V H S N G N T Y L E W Y L Q K P G Q S P K L L I Y K V S N	77										
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h12A11VL.prot	V P L T E G Q G T K L E I K	112										
bac01733	T P V T E G Q G T K L E I K	134										
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FIG. 5B

FIG. 6A

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FIG. 6B

R	I	T	I	S	K	N	T	V	Y	L	Q	M	N	S	L	R	A	D	T	A	V	Y	C	A
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RFTISkD	t	SKNT	V	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	C	A	R	v4.4		
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FIG. 6C

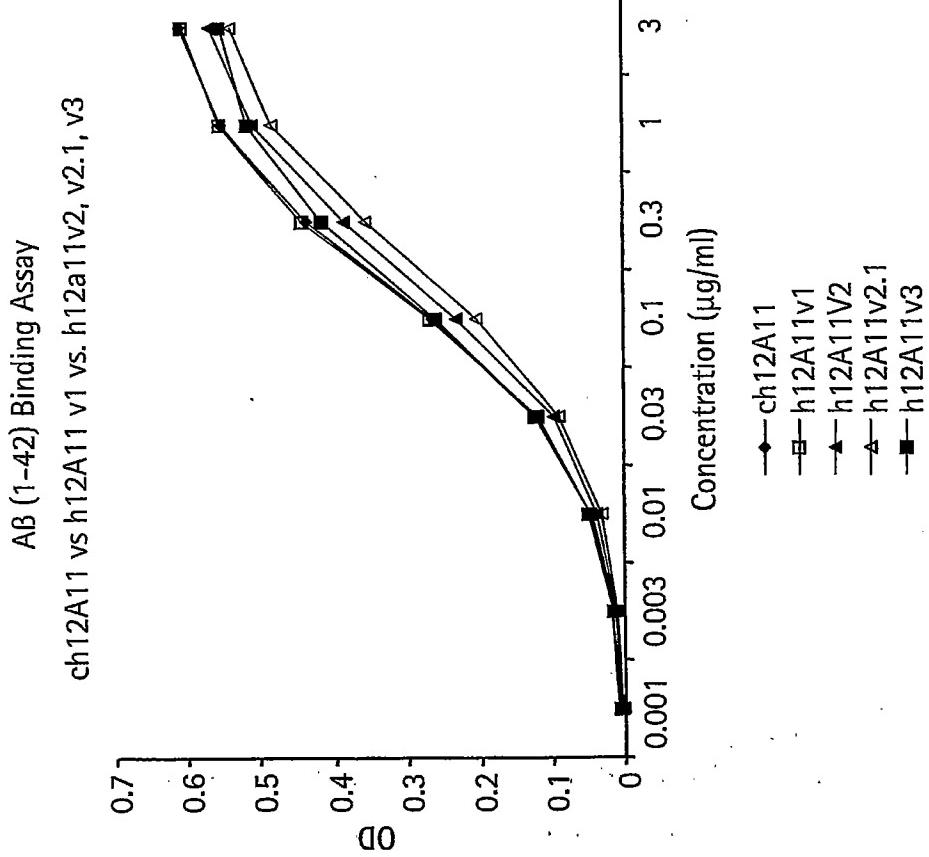
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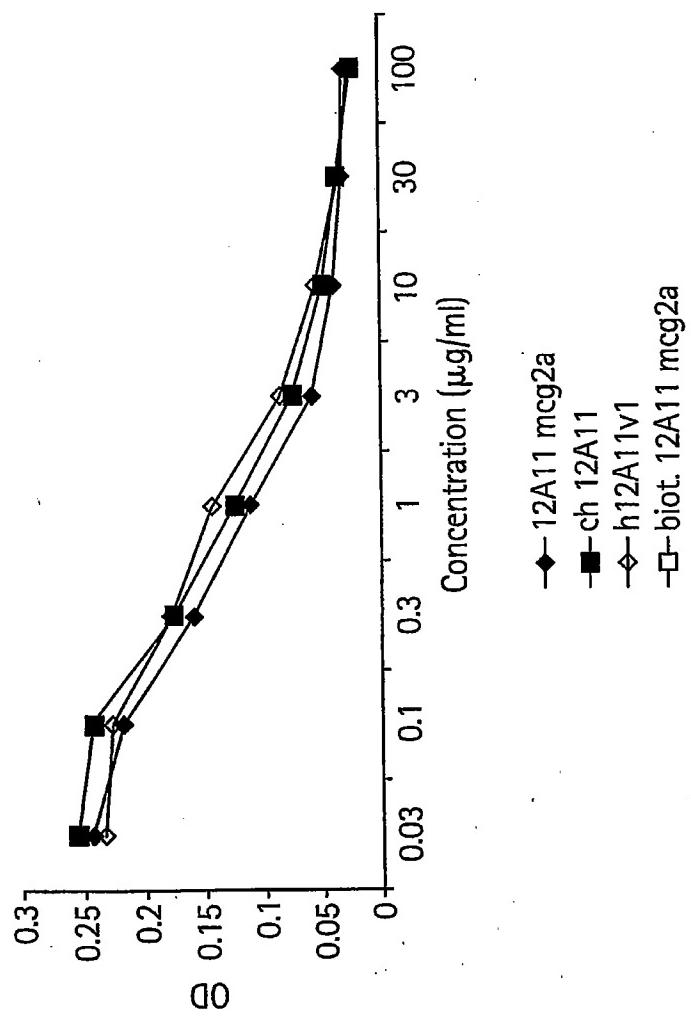
FIG. 7



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FIG. 8

Competition 12A11
12A11 mcg2a vs. ch12A11 vs. h12A11 v1



SEQUENCE LISTING

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1 5 10

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 Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys
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 1 5 10

ccc tca cag acc ctc agt ctg act tgt tct ttc tct ggg ttt tca ctg	144																																																		
Pro Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu																																																			
15	20	25		agc act tct ggt atg agt gta ggc tgg att cgt cag cct tca ggg aag	192	Ser Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Ser Gly Lys		30	35	40	45	ggt ctg gag tgg ctg gca cac att tgg tgg gat gat gat aag tac tat	240	Gly Leu Glu Trp Ile Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr		50	55	60		aac cca tcc ctg aag agc cgg ctc aca atc tcc aag gat acc tcc aga	288	Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg		65	70	75		aac cag gta ttc ctc aag atc acc agt gtg gac act gca gat act gcc	336	Asn Gln Val Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala		80	85	90		act tac tac tgt gct cga aga act act acg gct gac tac ttt gcc tac	384	Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr		95	100	105		tgg ggc caa ggc acc act ctc aca gtc tcc tca	417	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		110	115	120	
25																																																			
agc act tct ggt atg agt gta ggc tgg att cgt cag cct tca ggg aag	192																																																		
Ser Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Ser Gly Lys																																																			
30	35	40	45	ggt ctg gag tgg ctg gca cac att tgg tgg gat gat gat aag tac tat	240	Gly Leu Glu Trp Ile Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr		50	55	60		aac cca tcc ctg aag agc cgg ctc aca atc tcc aag gat acc tcc aga	288	Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg		65	70	75		aac cag gta ttc ctc aag atc acc agt gtg gac act gca gat act gcc	336	Asn Gln Val Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala		80	85	90		act tac tac tgt gct cga aga act act acg gct gac tac ttt gcc tac	384	Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr		95	100	105		tgg ggc caa ggc acc act ctc aca gtc tcc tca	417	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		110	115	120									
40	45																																																		
ggt ctg gag tgg ctg gca cac att tgg tgg gat gat gat aag tac tat	240																																																		
Gly Leu Glu Trp Ile Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr																																																			
50	55	60		aac cca tcc ctg aag agc cgg ctc aca atc tcc aag gat acc tcc aga	288	Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg		65	70	75		aac cag gta ttc ctc aag atc acc agt gtg gac act gca gat act gcc	336	Asn Gln Val Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala		80	85	90		act tac tac tgt gct cga aga act act acg gct gac tac ttt gcc tac	384	Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr		95	100	105		tgg ggc caa ggc acc act ctc aca gtc tcc tca	417	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		110	115	120																	
60																																																			
aac cca tcc ctg aag agc cgg ctc aca atc tcc aag gat acc tcc aga	288																																																		
Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg																																																			
65	70	75		aac cag gta ttc ctc aag atc acc agt gtg gac act gca gat act gcc	336	Asn Gln Val Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala		80	85	90		act tac tac tgt gct cga aga act act acg gct gac tac ttt gcc tac	384	Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr		95	100	105		tgg ggc caa ggc acc act ctc aca gtc tcc tca	417	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		110	115	120																									
75																																																			
aac cag gta ttc ctc aag atc acc agt gtg gac act gca gat act gcc	336																																																		
Asn Gln Val Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala																																																			
80	85	90		act tac tac tgt gct cga aga act act acg gct gac tac ttt gcc tac	384	Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr		95	100	105		tgg ggc caa ggc acc act ctc aca gtc tcc tca	417	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		110	115	120																																	
90																																																			
act tac tac tgt gct cga aga act act acg gct gac tac ttt gcc tac	384																																																		
Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr																																																			
95	100	105		tgg ggc caa ggc acc act ctc aca gtc tcc tca	417	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		110	115	120																																									
105																																																			
tgg ggc caa ggc acc act ctc aca gtc tcc tca	417																																																		
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser																																																			
110	115	120																																																	
120																																																			

<210> 4
<211> 139
<212> PRT
<213> Murine

<220>
<221> SIGNAL
<222> (1)...(19)

<400> 4
Met Asp Arg Leu Thr Thr Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr
-15 -10 -5
Val Leu Ser Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys
1 5 10
Pro Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu
15 20 25
Ser Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Ser Gly Lys
30 35 40 45
Gly Leu Glu Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr
50 55 60
Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg
65 70 75
Asn Gln Val Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala
80 85 90
Thr Tyr Tyr Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr
95 100 105
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110 115 120

<210> 5
<211> 429
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic

<400> 5
cmgmaagctt gcccacca tgaagttgcc tgtaggtctg ttgggtctga tgttctggat 60
tcctgcttcc agcagtgtatg ttttgatgac ccaaactcca ctctccctgc ctgtcagtct 120
tggagatcaa gcctccatct cttgcagatc tagtcagagc attgtacata gtaatggaaa 180
cacctactta gaatggtacc tgcagaaacc aggccagtc ccaaagctcc tgatctacaa 240
agtttccaac cgattttctg gggtcccaga cagggtcagt ggcagtggat cagggacaga 300
tttcacactc aagatcagca gagtgagggc tgaggatctg ggaattttatt actgctttca 360
aagttcacat gttcctctca cgttcggtgc tgggaccaag ctggagctga aacgtgagtg 420
gatccmgrp 429

<210> 6
<211> 445
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic

<400> 6
aagcttgcgg ccaccatgga caggcttaact acttcattcc tgctgctgat tgcctcgca 60
tatgtcttgtt cccaagttac tctaaaagag tctggccctg ggatattgaa gccctcacag 120
accctcagtc tgacttggc tttctctggg tttcactga gcacttctgg tatgagtgtt 180
ggctggattc gtcagccttc agggaaagggt ctggagtgcc tggcacacat ttgggtggat 240
gatgataaagt actataaccc atccctgaag agccggctca caatctccaa ggataacctcc 300
agaaaccagg tattcctcaa gatcaccagt gtggacactg cagataactgc cacttactac 360
tgtgctcgaa gaactactac ggctgactac tttgcctact ggggccaagg caccactctc 420
acagtctcct caggtgagtg gatcc 445

<210> 7
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v1 - VL region

<400> 7
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
20 25 30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Ser
85 90 95
Ser His Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 8
<211> 134
<212> PRT
<213> Homo sapiens

<220>
<221> SIGNAL
<222> (1)...(22)

<400> 8
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
-20 -15 -10
Ala Gln Pro Ala Met Ala Asp Val Val Met Thr Gln Ser Pro Leu Ser
-5 1 5 10
Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser
15 20 25
Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu
30 35 40
Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn
45 50 55
Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Thr
60 65 70
Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
75 80 85 90
Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Tyr Thr Phe Gly Gln Gly
95 100 105
Thr Lys Leu Glu Ile Lys
110

<210> 9
<211> 120
<212> PRT
<213> Homo sapiens

<220>
<221> SIGNAL
<222> (1)...(20)

<400> 9
Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Val Ser
-20 -15 -10 -5
Gly Ser Ser Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
1 5 10
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
15 20 25
Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys
30 35 40
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala
45 50 55 60
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
65 70 75
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
80 85 90
Cys Met Gln Ala Leu Gln Thr Pro
95 100

<210> 10
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> h12A11v1 - VH region

<400> 10
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 11
<211> 141
<212> PRT
<213> Homo sapiens

<220>
<221> SIGNAL
<222> (1)...(19)

<400> 11
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
-15 -10 -5
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
1 5 10
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
15 20 25
Ser Ser Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
30 35 40 45
Glu Trp Val Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
50 55 60
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
65 70 75
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
80 85 90
Tyr Tyr Cys Ala Arg Asp Arg His Ser Ser Ser Trp Tyr Tyr Gly Met
95 100 105
Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
110 115 120

<210> 12
<211> 137
<212> PRT
<213> Homo sapiens

<220>
<221> SIGNAL

<222> (1)...(19)

<400> 12
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
-15 -10 -5
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln
1 5 10
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
15 20 25
Ser Ser Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
30 35 40 45
Glu Trp Val Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
50 55 60
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
65 70 75
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
80 85 90
Tyr Tyr Cys Ala Arg Asp Ala Lys Leu Leu Met Leu Leu Ile Ser Gly
95 100 105
Ala Lys Gly Gln Trp Ser Pro Ser Leu
110 115

<210> 13
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v2

<400> 13
Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 14
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v2.1

<400> 14
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 15
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v3

<400> 15
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 16
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v4.1

<400> 16
 Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 17
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v4.2

<400> 17
 Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 18
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic h12A11v4.3

<400> 18
 Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 19

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v4.4

<400> 19

Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 20

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v5.1

<400> 20

Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 21
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v5.2

<400> 21
Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 22
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v5.3

<400> 22
Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
 65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
Gly Thr Thr Val Thr Val Ser Ser Val
 115 120

<210> 23
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v5.4

<400> 23
Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Thr Val Thr Val Ser Ser Val
115 120

<210> 24
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic h12A11v5.5

<400> 24
Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
35 40 45
Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
65 70 75 80
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95
Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 25
<211> 120
<212> PRT
<213> Artificial Sequence

<220>

<223> synthetic h12A11v5.6

<400> 25

Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 26

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A116.1

<400> 26

Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Phe Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Val
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Thr Thr Ala Asp Tyr Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 27

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic h12A11v6.2

<400> 27

Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Phe Ser Gly Phe Thr Leu Ser Thr Ser
 20 25 30
 Gly Met Ser Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Val Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Thr Leu
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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 35 40 45
 Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val
 65 70 75 80
 Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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ctccagatga acagtctgcg ggctgaagat actgccgtgt actactgtgc tcgaagaact 360
actaccgctg actactttgc ctactgggc caaggcacca ctgtcacagt ctccctca 417

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17/00, C12P 21/08, A61K 39/395, 39/40, 39/42

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Enfield, EN1 3BD (GB). **BARD, Frédérique** [US/FR];
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(21) International Application Number:
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(74) Agents: **MILASINCIC, Debra, J.** et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).

(22) International Filing Date: 1 June 2004 (01.06.2004)

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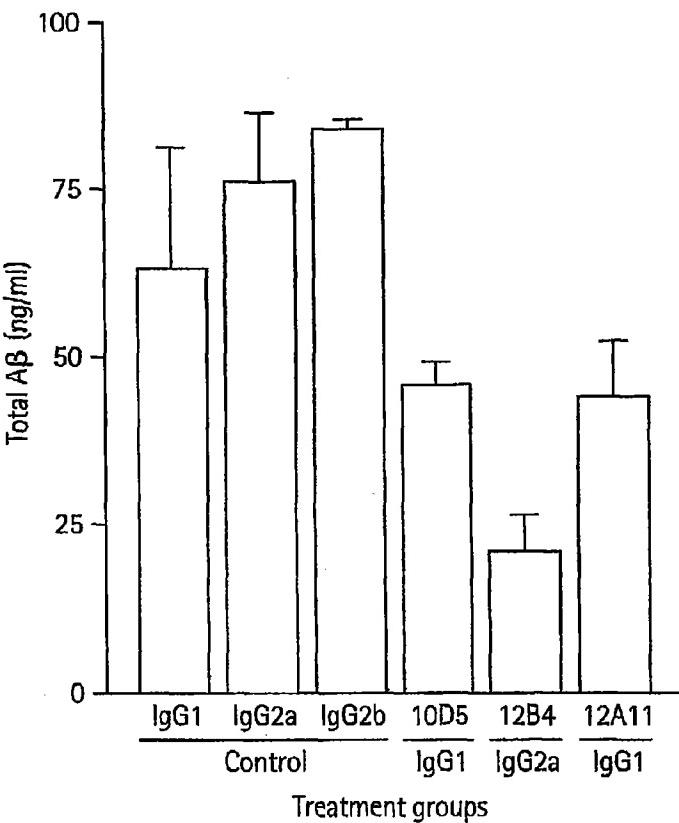
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(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH,

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[Continued on next page]

(54) Title: HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE



(57) Abstract: The invention provides improved agents and methods for treatment of diseases associated with amyloid deposits of A β in the brain of a patient. Preferred agents include antibodies, e.g., humanized antibodies.

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GM, KE, LS, MW, MZ, NA, SD, SL, SŽ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
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SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/17514

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 16/00, 17/00; C12P 21/08; A61K 39/395, 39/40, 39/42
US CL : 530/387.1, 387.2, 387.3, 388.1, 388.15; 424/130.1, 141.1, 142.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/387.1, 387.2, 387.3, 388.1, 388.15; 424/130.1, 141.1, 142.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,750,324 B1 (SCHENK et al) 15 June 2004 (15.06.2004), entire document.	1-10, 34-44, 121-124
A	US 6,743,427 B1 (SCHENK) 01 June 2004 (01.06.2004), entire document.	1-10, 34-44, 121-124
A	SOLOMON, B. Immunological approaches as therapy for Alzheimer's disease. Expert Opin Biol Ther. December 2002, Vol. 2, No. 8, pages 907-917, entire document.	1-10, 34-44, 121-124
A	HOLTZMAN, D.M. et al. Abeta immunization and anti-Abeta antibodies: potential therapies for the prevention and treatment of Alzheimer's disease. Advanced Drug Delivery Reviews Vol. 54, No. 12, pages 1603-1613, see Figure 1.	1-10, 34-44, 121-124-10, 34-44, 121-124
A	DODEL, R. C. et al. Immunotherapy for Alzheimer's disease. Lancet Neurology April 2003, Vol. 2, No. 4, pages 215-220, see Figure 2.	1-10, 34-44, 121-124

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

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Authorized officer
Christopher J. Nichols, Ph.D.
Telephone No. 703-308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/17514

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 11-23
because they relate to subject matter not required to be searched by this Authority, namely:
Please See Continuation Sheet

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-10, 34-44 and 121-124

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/17514

Box II Observations where certain claims were found unsearchable I. because they relate to subject matter not required to be searched by this Authority, namely:

Claims 11-23 and 78-83 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description. The description does not disclose the claimed invention in a manner sufficiently clear and complete for the claimed invention to be carried out by a person skilled in the art because: claims 11-23 require use of "analysis of a three-dimensional model" which is not disclosed or taught in the instant specification. This also represents non-statutory matter as it is a mental step and not the physical manipulation of matter.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1 Claim(s) 1-10, 24-49, and 121-124, drawn to humanized antibody 12A11, compositions, and methods of use thereof.

Group 2 Claim(s) 50-65, drawn to an isolated polypeptide and variants thereof.

Group 3 Claim(s) 66-72 and 75, drawn to an isolated nucleic acid, vectors, and host cells comprising same.

Group 4 Claim(s) 73-74, drawn to a transgenic animal.

Group 5 Claim(s) 76-77, drawn to a method of producing an antibody.

Group 6 Claim(s) 84-85, drawn to a method of treating an amyloidogenic disease comprising administering a nucleic acid molecule.

Group 7 Claim(s) 86-120, drawn to a method for reducing neuritic burden comprising administering a humanized immunoglobulin.

The inventions listed as Groups 1-7 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group 1 is drawn to the special technical feature of humanized antibody 12A11, which is not required by any of the other groups.

Group 2 is drawn to the special technical feature of an isolated polypeptide, which is not required by any of the other groups.

Group 3 is drawn to the special technical feature of an isolated nucleic acid, vectors, and host cells comprising same, which is not required by any of the other groups.

Group 4 is drawn to the special technical feature of a transgenic animal, which is not required by any of the other groups.

Group 5 is drawn to the special technical feature of producing an antibody, which is not required by any of the other groups.

Group 6 is drawn to the special technical feature of gene therapy, which is not required by any of the other groups.

Group 7 is drawn to the special technical feature of passive immunization therapy, which is not required by any of the other groups.

Claims 11-23 and 78-83 are unsearchable under PCT Rule 6.4(a).

INTERNATIONAL SEARCH REPORT

International application No. PCT/US04/17514

Continuation of B. FIELDS SEARCHED Item 3:
WEST (USPT, PGPUBS, USOCR, JPO, EPO, DERWENT); NCBI (PUBMED); STN (BIOSCIENCE)
12A11, 10D5, chimeric, humanized, antibody, Abeta, Alzheimers disease, passive immunization